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PHARMACOKINETICS AND MOLECULAR DOCKING-BASED INVESTIGATION OF BIOACTIVE COMPOUNDS FROM *Curcuma longa* TARGETING KEY PROTEINS IN LOW BACK PAIN MANAGEMENT

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Abstract

Low back pain (LBP) is a leading health issue throughout the world. It results from several contributing factors including muscle strain, structural problems, arthritis, and disk injuries. Many times, LBP is linked with side effects of conventional pharmacological treatments including NSAIDs and opioids, calling for the safer alternatives. Curcumin and demathoxycurcumin are bioactive natural compounds of Curcuma longa and both of these compounds are known to reduce inflammation and ease pain. This study is designed to investigate the role of these compounds in the treatment the LBP as alternatives of synthetic medicines. This study used pharmacokinetics analysis and molecular docking to test how well these compounds could be used as medicines to target the key proteins involved in LBP. To study pharmacokinetics characteristics of these natural compounds the online servers including Swiss ADME, STOPTOX and pkCSM, were used. While, the interaction of these compounds with key protein involved in LBP was checked through Maestro 12.5. Results of molecular docking analysis showed significant interaction with residues of these key proteins. Furthermore, this suggests that these compounds can play a significant role in controlling inflammatory pathways in LBP. The ADMET analysis suggested good drug-likeness, demonstrating excellent gastrointestinal absorption, low toxicity, and conformity with Lipinski's rule of five. These compounds showed low blood brain barrier permeability, though, with would limit the central nervous system effects. Furthermore, NMA analysis showed stable bindings between these two compounds and target proteins suggesting that both of these compounds could be the useful natural alternatives for controlling LBP.

INTRODUCTION

Low Back Pain (LBP) is a common health issue all around the world, and it affected 619 million persons globally in 2020 (Xu *et al.*, 2025). According to medical assumptions, this figure is expected to increase to 843 million instances by 2050 (Jurak *et al.*, 2023). The global data confirms that LBP as the main disability-inducing illness causing significant social and financial effects (Eshraghi *et al.*, 2022). LBP results from several contributing factors, including muscle strain, structural problems, arthritis, and disk injuries (Abdelbasset & Sulieman, 2022). Pharmacological therapy, nonsteroidal anti-inflammatory medications (NSAIDs) and opioids are used to treat low back pain, using such therapies causes different side effects, including gastrointestinal difficulties and dependency potential (Anderson & Shaheed, 2022; Alorfi, 2023). The search for remedies that are both efficient and preserve greater safety features drives increasing curiosity about alternative medicines.

Traditional medicine has demonstrated the use of *Curcuma longa* (turmeric) to help patients with pain reduction. *Curcuma longa* mostly produces curcumin and demethoxycurcumin that have great anti-inflammatory effects (Memarzia *et al.*, 2021; Jain *et al.*, 2025). In drug discovery, toolkit development depends much on the molecular docking computational techniques (Siddiqui *et al.*, 2025). Scientists can guess how bioactive compounds might interact with certain protein targets using this method, which helps them find new possible therapies (Muhammed & Aki-Yalcin, 2024). Researchers use molecular docking to identify the chemical interactions of plant based bioactive compounds, and important LBP pathophysiology proteins (Lu *et al.*, 2024). This allows them to investigate the potential alternative medical treatment efficacy of these substances.

Modern scientists have investigated how various curcumin derivatives bind to and interact with different protein targets using molecular docking methods. Strong linkages between these compounds and protein targets that would explain their therapeutic effect were found through the use of altered curcumin structures in research (Sharifi-Rad *et al.*, 2020). Combining computational methods with conventional medical knowledge effectively accomplishes the development of new therapeutic compounds (Li *et al.*, 2022). By means of molecular docking studies, researchers can accelerate the process of improving pharmaceutical chemicals extracted from medicinal plants. Safer therapeutic choices resulting from this can be applied to treat LBP (Farihi *et al.*, 2023; Rajalekshmi & Agrawal, 2024). The goal of this study is to investigate how several bioactive compounds from *Curcuma longa*, bind to and interact with key proteins that control LBP. Furthermore, the aim is to study the safety of these compounds in medicine.

2. Methodology

2.1 Study Design

This research employed theoretical screening study using online computational pharmacokinetics and molecular docking looked into the bioactive compounds' curcumin and demethoxycurcumin from Curcuma longa, as well as the proteins that cause low back pain.

2.2 Selection of Bioactive Compounds

Curcumin and demethoxycurcumin were selected for this study as both of these bioactive compounds are known for their anti-inflammatory and reducing pain abilities (Razavi *et al.*, 2021).

2.3 Selection and Preparation of Target Proteins

We chose the target proteins because they played crucial roles in LBP pathophysiology, pain modulation, and inflammation regulation. For

the study, Cyclooxygenase-2 (COX-2) (PDB ID: 5IKQ), Tumor Necrosis Factor-alpha (TNF-α) (PDB ID: 2AZ5), and Interleukin-6 (IL-6) (PDB ID: 1ALU) were used as proteins. Pertinently, scientists selected these proteins because they substantially participate in inflammatory processes and pain signals.

2.4 Molecular Docking Procedure

Molecular docking was conducted using Schrödinger's Glide module in Maestro 12.5, which allows high-precision ligand-receptor docking by analyzing binding affinities and molecular interactions. The docking workflow followed three major steps: ligand preparation, receptor grid generation, and docking simulation (Um *et al.*, 2022).

2.5 ADMET and Drug-Likeness Prediction

The pharmacokinetic properties of curcumin and demethoxycurcumin in this study were performed by using Swiss ADME, STOPTOX, and pkCSM tools. The online tools use molecular descriptor analysis to assess the drug's similarity, bioavailability, metabolization, and toxicity risks. The drug-likeness analysis used Lipinski's Rule of Five to check the chosen compounds for drug development parameters, such as lipophilicity (from 5 to less than 5), hydrogen bond donors (not more than 5), and hydrogen bond acceptors (not more than 10). The compounds' weight had to be less than 500 Daltons. In a study, the BBB was penetrated, the drug was absorbed in the digestive tract, and the cytochrome P450 metabolism was analyzed to find out what effect it had on the whole body and how stable the metabolism was.

Results

3.1 Characteristics of Selected Compounds

Table 1 provides detailed information on the selected bioactive compounds from *Curcuma longa* (Turmeric) and *Boswellia serrata* (Frankincense), including their molecular formulas, PubChem CID, and SMILES notation. Curcumin and Demethoxycurcumin, derived from Turmeric, are known for their potent anti-inflammatory properties, while β-Boswellic Acid and Acetyl-11-Keto-β-Boswellic Acid (AKBA) from Frankincense exhibit strong analgesic and anti-inflammatory effects. These compounds have been investigated as potential natural alternatives to conventional drugs for managing low back pain.

Table 1: Information of selected bioactive compounds

Plant Name	Bioactive Compound	PubChem CID	SMILES Notation
Turmeric	Curcumin (C21H20O6)	969516	COC1=C(C=CC(=C1)/C=C/C(=O)CC(=O)/C=C/C2=CC(=C(C=C2)O)OC)O
Turmeric	Demethoxycurcumin (DMC) (C20H18O5)	5469424	COC1=C(C=CC(=C1)/C=C/C(=O)CC(=O)/C=C/C2=CC=C(C=C2)O)O

3.2 ADMET and Drug-Likeness Prediction

3.2.1. Swiss ADME properties

The heatmap provides a detailed visualization of the ADME (Absorption, Distribution, Metabolism, and Excretion) properties of the phytocompounds Curcumin and Demethoxycurcumin. The y-axis represents various physicochemical properties, while the x-

axis lists the two phytocompounds. The color gradient, ranging from blue to red, indicates the magnitude of each property, with higher values shifting towards red and lower values appearing in blue. This representation allows for a quick assessment of the numerical trends across multiple pharmacokinetic parameters.

Among the properties displayed, molecular weight (MW) is one of the most significant, indicating the overall size of each compound. Other key attributes include the number of heavy atoms, aromatic heavy atoms, and fraction Csp^3, which describe the structural complexity and hybridization state of the molecules. Properties such as topological polar surface area (TPSA) and hydrogen bond donors/acceptors influence solubility and permeability, critical for predicting absorption and bioavailability. Additionally, various logP values (iLOGP, XLOGP3, WLOGP, and MLOGP) reflect lipophilicity, a crucial factor affecting membrane permeability and drug-likeness.

Further, the heatmap presents essential descriptors such as solubility (ESOL Log S and Silicos-IT LogSw), skin permeability (log Kp), and different drug-likeness rules like Lipinski, Ghose, Veber, and Egan violations. The bioavailability score provides an estimation of the compound's potential to be orally active, while structural alerts such as Brenk and PAINS flags help assess the likelihood of toxicity or reactivity. Synthetic accessibility scores indicate how challenging a compound might be to synthesize, which is important in drug development. This heatmap serves as an insightful tool for analyzing the pharmacokinetic and physicochemical properties of the given phytocompounds (Figure 1).

The table describes the ADME (Absorption, Distribution, Metabolism, and Excretion) characteristics of curcumin and demethoxycurcumin. The high gastric absorption level of these compounds indicates they will have excellent absorption properties after oral intake. The blood-brain barrier acts as an impediment, preventing both phytocompounds from reaching CNS tissues. Both phytocompounds don't work as P-glycoprotein substrates, so there is low active cell efflux. This helps cells stay in place (Table 2). Demethoxycurcumin can change how drugs are broken down by CYP1A2 because it blocks this enzyme. Curcumin, on the other hand, does not change how CYP1A2 works. These chemicals can interact with drugs because they stop the main cytochrome P450 enzymes CYP2C9 and CYP3A4 from working. The data show that these substances don't stop CYP2C19 or CYP2D6 from working. This means that there is less chance that drugs will interact with these enzyme pathways and cause problems.

The skin doesn't let much curcumin or demethoxycurcumin through because their log Kp values are negative, at -6.28 cm/s and -6.01 cm/s, respectively. The poor skin permeability values obtained from these compounds demonstrate that topical delivery through skin might not work effectively. Results from the research illustrate the pharmacokinetic properties of both compounds and help define their conduct in drugs and clinical applications (Table 2).

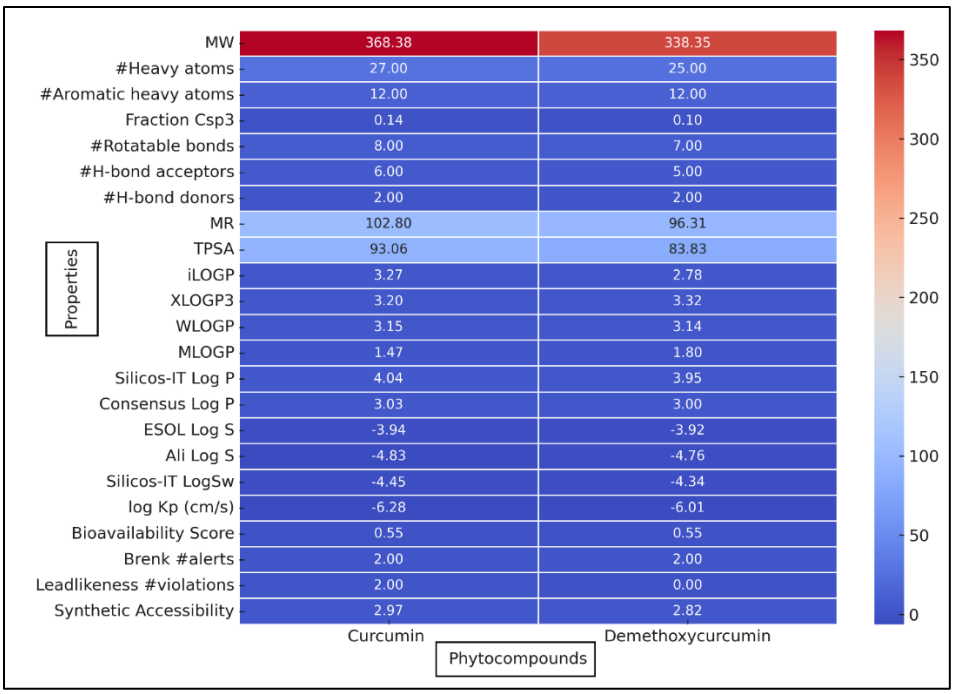

Figure1: Heatmap of ADME properties of Curcumin and Demethoxycurcumin, illustrating various pharmacokinetic parameters

Table 2: Pharmacokinetic Properties of Curcumin and Demethoxycurcumin

ADME properties	Curcumin	Demethoxycurcumin
GI absorption	High	High
BBB permeant	No	No
P-gp substrate	No	No
CYP1A2 inhibitor	No	Yes
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	Yes	Yes
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	Yes	Yes
Log Kp (skin permeation)	-6.28 cm/s	-6.01 cm/s

The BOILED-egg model shows how curcumin and demethoxycurcumin get into the brain from the mouth and through the digestive tract. The white area shows that the substance can be absorbed by humans and is bioavailable when taken by mouth. Next to it is a yellow area that shows how well the substance can pass through the blood-brain barrier. Curcumin and demethoxycurcumin can be absorbed in the HIA zone, but they can't cross the BBB very easily, which means they can get into the GI tract but not the brain. A red circle inside both compounds shows the status of the P-glycoprotein (PGP+) substrate and their active efflux properties, which change how available they are in body tissues. The accompanying graphical plots illustrate six physical properties, namely LIPO for lipophilicity, POLAR for polarity, SIZE

for molecular size, FLEX for flexibility, INSOLU for insolubility, and INSATU for unsaturation. The structural models verify that the two molecules share similarities but show slight differences in their functional components. The bioavailability of curcumin and demethoxycurcumin is similar because they are both absorbed slowly and don't get too far into the central nervous system.

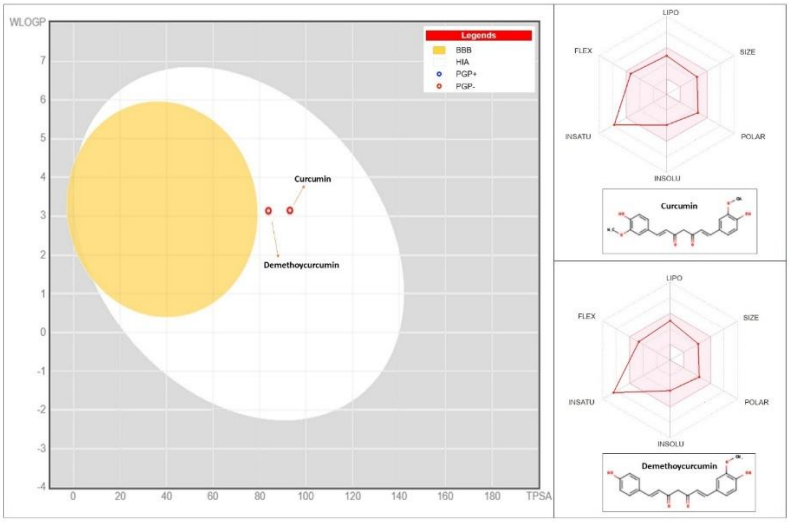

Figure 2: BOILED-Egg model analysis of Curcumin and Demethoxycurcumin, showing their positions in the gastrointestinal absorption (HIA) and blood-brain barrier (BBB) permeability regions. The radar plots illustrate their physicochemical properties, while molecular structures provide structural insights.

3.2.2. pkCSM pharmacokinetic analysis

The PkCSM model analyzed the pharmacokinetic behavior of curcumin and demethoxycurcumin to investigate their ADMET properties, which include absorptive, distributive, metabolic, excretive, and toxicological aspects. The rates at which both compounds absorbed water were moderate to low, but demethoxycurcumin was slightly more soluble than curcumin. Because it did better in the Caco-2 permeability tests, demethoxycurcumin was better at being absorbed by the intestines than curcumin. Both compounds demonstrated strong human intestinal absorption, though demethoxycurcumin demonstrated superior absorption effects. The skin penetration rate was poor for each compound. The results showed that curcumin and demethoxycurcumin bind to and block P-glycoprotein I and II, which suggests that they work together in a way that affects drug efflux mechanisms.

A low volume of distribution in human patients indicates minimal tissue spread for these compounds. In the lab, tests showed that curcumin and demethoxycurcumin didn't have many free substances in plasma, which showed that they bind strongly to plasma proteins. The blood-brain barrier let some of these compounds through, but demethoxycurcumin was able to get through slightly better than the other compound. Both agents presented restricted entry into the central nervous system, similar to their other behavioral barriers.

The tests revealed that CYP3A4 can metabolize curcumin and demethoxycurcumin, suggesting a potential hepatic breakdown by this enzyme. However, both compounds failed to serve as substrates for CYP2D6. When tested against CYP1A2, CYP2C19, CYP2C9, and CYP3A4, the

compounds blocked enzymes, but not when tested against CYP2D6. The predicted total excretion levels of these substances revealed low clearance rates, while demethoxycurcumin showed marginally higher clearance than curcumin. The assessment indicated that renal OCT2 would not take up these compounds as substrates, resulting in limited renal excretion by this transporter.

computational tests using Ames toxicity showed that these compounds did not cause mutation, which indicates they harbor no mutagenic properties. Laboratory results found curcumin could tolerate human doses slightly better than its derivative compound demethoxycurcumin. The tests did not find any hERG I inhibitory activity between the compounds. However, demethoxycurcumin showed that it had inhibitory effects on hERG II, which could mean that the compounds could be harmful to the heart. Tests demonstrated that both compounds produced no harmful effects to liver tissue and showed zero sensitivity to skin activation potentials. Overall, both curcumin and demethoxycurcumin have good pharmacokinetic properties, meaning they are easily absorbed in the intestines and don't cause many health problems. The difficulty of these compounds passing through both the blood-brain barrier and CNS, combined with possible metabolic enzymatic interactions, may affect their availability and therapeutic performance (Table 3).

Table 3: pkCSM pharmacokinetic parameters of the selected ligands

Property	Model Name	Predicted Value		Unit
		Curcumin	Demethoxycurcumin	
Absorption	Water solubility	-4.01	-3.566	Numeric (log mol/L)
	Caco2 permeability	-0.093	1.023	Numeric (log Papp in 10^{-6} cm/s)
	Intestinal absorption (human)	82.19	91.393	Numeric (% Absorbed)
	Skin Permeability	-2.764	-2.768	Numeric (log Kp)
	P-glycoprotein substrate	Yes	Yes	Categorical (Yes/No)
	P-glycoprotein I inhibitor	Yes	Yes	Categorical (Yes/No)
	P-glycoprotein II inhibitor	Yes	Yes	Categorical (Yes/No)
Distribution	VDss (human)	-0.215	-0.075	Numeric (log L/kg)
	Fraction unbound (human)	0	0	Numeric (Fu)
	BBB permeability	-0.562	-0.337	Numeric (log BB)
	CNS permeability	-2.99	-2.458	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	No	Categorical (Yes/No)
	CYP3A4 substrate	Yes	Yes	Categorical (Yes/No)
	CYP1A2 inhibitior	Yes	Yes	Categorical (Yes/No)
	CYP2C19 inhibitior	Yes	Yes	Categorical (Yes/No)
	CYP2C9 inhibitior	Yes	Yes	Categorical (Yes/No)

	CYP2D6 inhibitior	No	No	Categorical (Yes/No)
	CYP3A4 inhibitior	Yes	Yes	Categorical (Yes/No)
Excretion	Total Clearance	-0.002	0.026	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	No	Categorical (Yes/No)
	Max. tolerated dose (human)	0.081	-0.12	Numeric (log mg/kg/day)
	hERG I inhibitor	No	No	Categorical (Yes/No)
	hERG II inhibitor	No	Yes	Categorical (Yes/No)
	Hepatotoxicity	No	No	Categorical (Yes/No)
	Skin Sensitisation	No	No	Categorical (Yes/No)

3.2.3. STOPTOX Acute toxicity test

The STOPTOX acute toxicity test results disclose toxicological data about curcumin and demethoxycurcumin through their assessment of acute inhalation toxicity and dermal exposure and oral toxicities and their effects on skin and eyes. Laboratory experiments demonstrated that curcumin and demethoxycurcumin demonstrated no toxic effects during tests for acute inhalation toxicity as well as dermal and oral toxicity. The prediction reliability for acute inhalation toxicity based on toxicological analysis reached 53% for curcumin and 57% for demethoxycurcumin, which signifies moderate prediction accuracy. Both compounds demonstrated better confidence levels for acute oral toxicity tests since curcumin achieved 72% and demethoxycurcumin obtained 75%, agreeing on their non-toxic properties during ingestion. An evaluation of acute dermal toxicity showed that curcumin and demethoxycurcumin were safe for human skin, with confidence levels of 59% for curcumin and 55% for demethoxycurcumin. Studies and evaluation standards that reached 88% and 86% confidence levels showed that both curcumin and demethoxycurcumin were safe for the eyes to be exposed to. The likelihood data indicates both compounds would not produce substantial eye irritations. Both compounds received sanitizer classifications during the skin sensitization tests, indicating possible skin sensitization effects. The predicted accuracy for classifying curcumin and demethoxycurcumin was about 60% for curcumin and 50% for demethoxycurcumin. When exposed repeatedly or for long periods of time, certain individuals might develop allergic skin conditions or show sensitivity reactions. Evaluation results show curcumin alongside demethoxycurcumin to have no skin irritation effects, so their exposure on human skin surfaces is expected to be safe. The evaluation performed with a 90% confidence level indicated high safety reliability for both compounds in terms of their response to skin irritation. According to the STOPTOX acute toxicity test, curcumin and demethoxycurcumin are mostly safe. They don't hurt when breathed in, eaten, or applied to the skin, and they also don't hurt when tested for eye irritation and skin irritation. People with sensitive skin, along with those who regularly encounter these compounds, should exercise caution because these substances demonstrate sensitization properties.

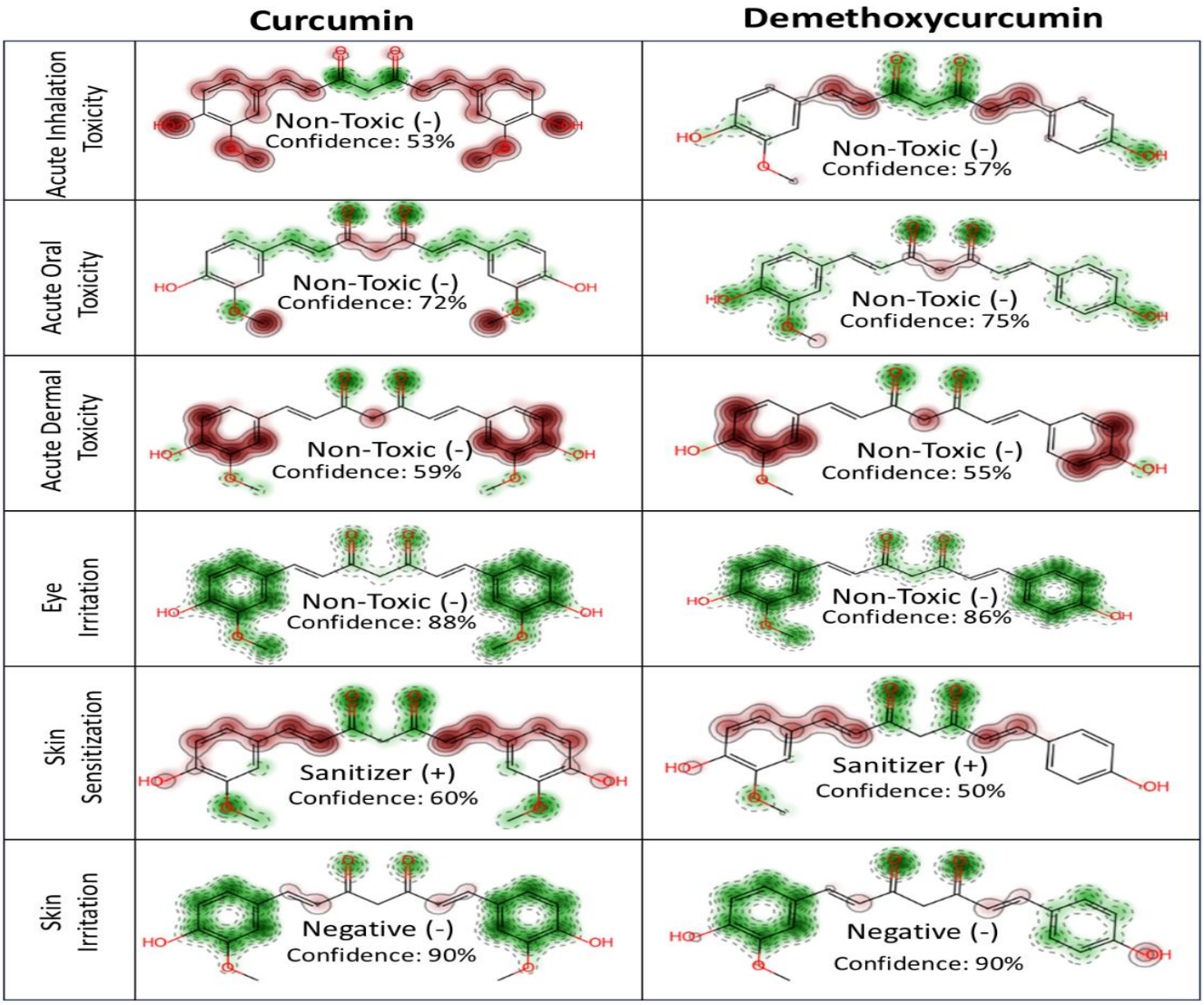

Figure 3: STOPTOX acute toxicity predictions for curcumin and demethoxycurcumin, assessing various toxicological endpoints including acute inhalation, oral, and dermal toxicity, eye irritation, skin sensitization, and skin irritation

3.2 Molecular Docking Results

The molecular docking analysis shows that curcumin and demethoxycurcumin from Curcuma longa could be used as natural alternatives to prescription drugs to treat low back pain because they interact strongly with key proteins in the pain and inflammation pathways. In tests on COX-2 (PDB ID: 5IKQ) for pain and inflammation management, demethoxycurcumin (-5.92 kcal/mol) binds more strongly than curcumin (-3.3 kcal/mol). Demethoxycurcumin and curcumin may be good natural alternatives to COX-2 inhibitors because they are safe for humans, come from plants, and help reduce inflammation. However, their binding affinity levels remain lower than the reference compound (-10.30 kcal/mol). The binding scores for curcumin (-4.98 kcal/mol) and demethoxycurcumin (-4.97 kcal/mol) to TNF-α

(PDB ID: 2AZ5) were almost the same as the reference compound (-5.26 kcal/mol). These natural agents possess properties that suggest they can control TNF-α activity, thus making them suitable candidates for anti-inflammatory therapy.

When IL-6 (PDB ID: 1ALU) was looked at as an inflammatory mediator, curcumin (-3.40 kcal/mol) formed a stronger binding interaction than demethoxycurcumin (-3.20 kcal/mol), though both scores were close to those of the reference compound (-3.80 kcal/mol). There is evidence that the compounds can change the activity of IL-6, which in turn lowers the inflammatory response and pain. Curcumin and demethoxycurcumin can bind to inflammatory targets at levels that are lower than those found in most drugs. This suggests that they may be safe natural ways to treat inflammatory pain, especially low back pain. These substances are good candidates for further research into natural pain management because they have therapeutic potential, are safe, and can be used for a long time (Table 4).

3.3 Protein-Ligand Interaction Analysis

The molecular docking results in Figure 4 show how Curcuma longa and Boswellia serrata bioactive compounds bind to the low- back pain management proteins cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). The figure comprises three rows, each representing a different target protein. Three columns illustrate the docking interaction perspectives, including active site ligand binding, surface representation, and 2D interaction views. The first row shows demethoxycurcumin (DMC) interacting with COX-2. According to the molecular surface overview, COX-2 binds inside the active site. A binding pocket analysis shows that LEU534 and PHE529 and GLY533 amino acid residues interact in important ways, which shows that the compound can help reduce inflammation. The two-dimensional interaction diagram shows how hydrogen bonding and hydrophobic interactions keep the ligand in place in the active site.

The surface model in this row demonstrates how curcumin inserts itself into TNF-α's binding area. A closer look at the curcumin binding site shows that it is linked to key protein residues GLY121, VAL123, and TYR151, which could be proof of mechanisms that stop the TNF-α inflammatory pathway. The 2D interaction map shows the hydrogen bonds and hydrophobic bonding elements that help keep the binding process stable.

The third docking arrangement demonstrates how curcumin fits into the IL-6 binding site. The study found important molecular links between ARG162 and GLN175 and ARG179, which suggests that they may stop IL-6 signaling. The 2D picture shows that the stabilizing interactive forces that might make the compound's anti-inflammatory effects stronger are real. The binding analysis in Figure 1 shows that these bioactive compounds interact meaningfully with COX-2, TNF-α, and IL-6. This suggests that they could be used as natural alternatives to treat low back pain. The docking findings validate these compounds' capacity to regulate significant inflammatory processes, but further clinical trials are required to assess their efficacy in pain management (Figure 4).

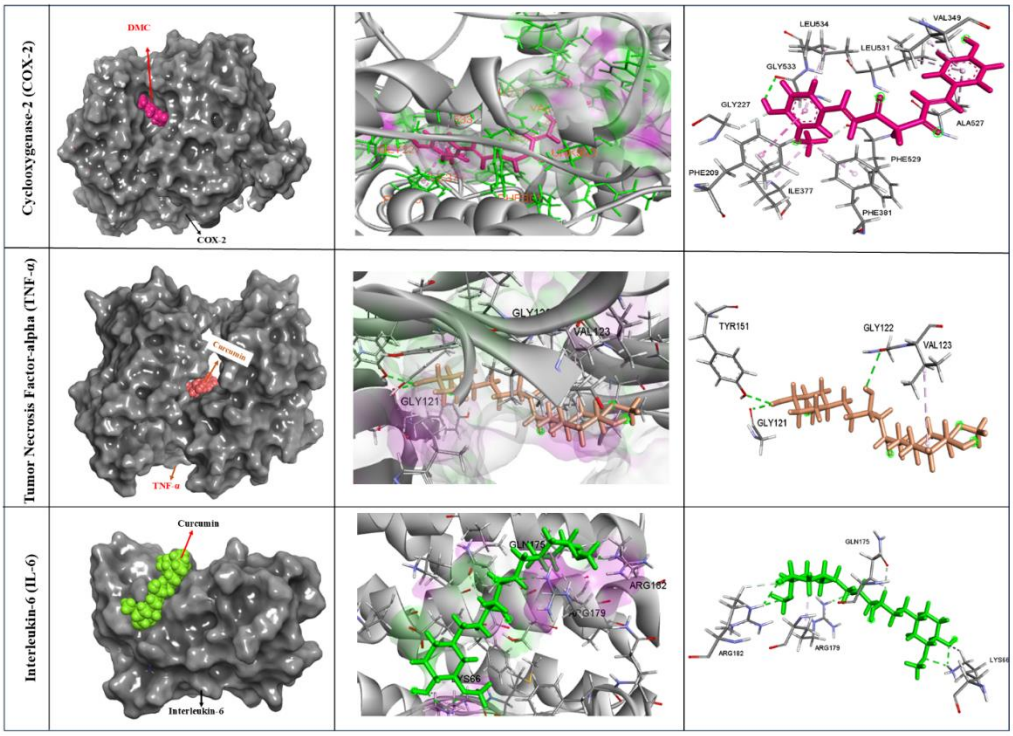

Figure 4: Protein-Ligand Interaction Analysis of COX-2, TNF-α, and IL-6 with Bioactive Compounds.

Table 4: Molecular Docking Scores of Bioactive Compounds from *Curcuma longa* Against Key Proteins Involved in Low Back Pain Management

Target Protein	PDB ID	Curcumin (kcal/mol)	Demethoxycurcumin (kcal/mol)	Reference compound
COX-2	5IKQ	-3.3	-5.92	-10.30
TNF-α	2AZ5	-4.98	-4.97	-5.26
IL-6	1ALU	-.3.40	-3.20	-3.80

3.4. Normal Mode Analysis (NMA)

Researchers can figure out important dynamic patterns of the protein-ligand structure by looking at the COX-2 (PDB ID: 5IKQ) and demethoxycurcumin complex normal mode analysis. The deformable protein regions of the complex structure point to flexible binding sites that promote both compound integration and protein shape alterations. Research evidence confirms this discovery as the data peaks indicate mobile protein regions that guide the analysis of binding responses between proteins and ligands. Experimental PDB data confirms the stability of the complex by comparing NMA predicted fluctuations to actual experimental measurements between the two data sets. When the complex's eigenvalue goes down, it moves more efficiently, which means there are good binding sites for demethoxycurcumin on COX-2. In variance analysis, larger ranges of sequential indices show that protein structure moves in big ways. Covariance maps help us understand how proteins work and how stable they are by showing the patterns of relationships between residues that interact with each other based on how they move together and against each other. The elastic network model backs up the research results by showing that there are strong interatomic links

that help keep the structure stable. Researchers have found that the COX-2-Demethoxycurcumin combination keeps the structure intact while letting parts move. This shows that it can be used as a natural COX-2 inhibitor to treat low back pain (Figure 5).

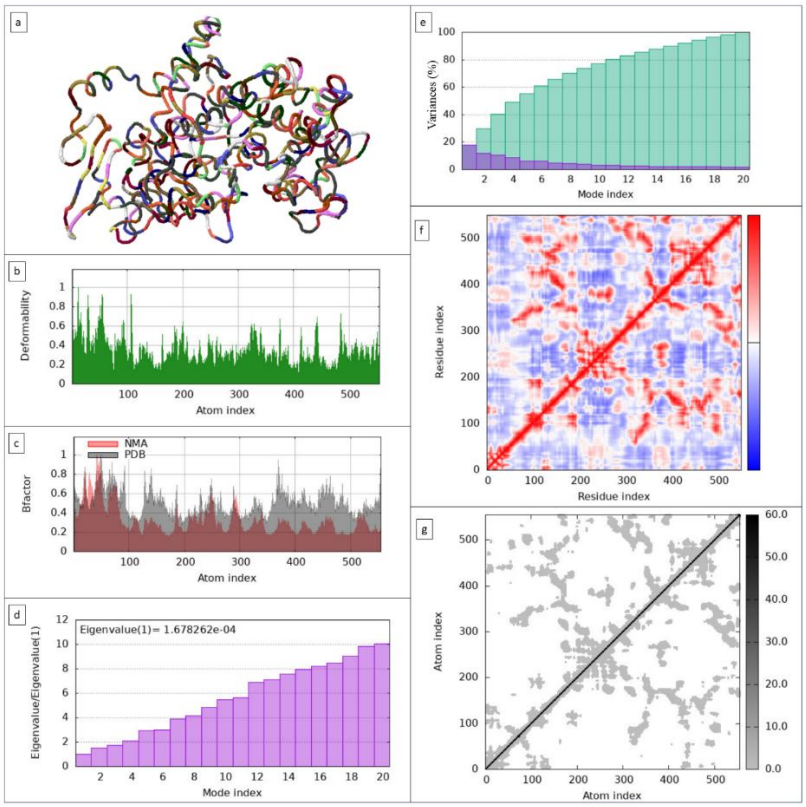

Figure 5: Normal Mode Analysis (NMA) of the COX-2-Demethoxycurcumin Complex. (a) Normal mode representation showing collective motions. (b) Deformability plot highlighting flexible regions. (c) B-factor analysis comparing NMA-predicted fluctuations with experimental PDB data. (d) Eigenvalue analysis indicating molecular motion efficiency. (e) Variance distribution of normal modes contributing to structural flexibility. (f) Covariance map depicting correlated and anti-correlated residue motions. (g) Elastic network model illustrating atomic interaction strength and stability.

This test, called Normal Mode Analysis, shows how TNF-α (PDB ID: 2AZ5) and curcumin work together. It also shows how both proteins' movement and stability change when they are together. In Figure 6a, the graph shows how protein-ligand interactions move by using specific color patterns to show different flexible areas. Figure 6b shows the protein deformability plot that indicates bendable areas that likely work in binding and regulating protein behavior. Figure 6c shows B-factor analysis shows that when curcumin binds, it strengthens residues in important binding areas and changes how they move between different areas.

The graph 6d displays eigenvalue results to measure protein flexibility and also reveals lower energy shapes of movement. This reveals what proportion of the normal positions impacts structural changes (Figure 6e). Figure 6f displays how residues relate with one another as either

supportive or in opposition through their movements in the signaling networks. Figure 6g represents the elastic network model used to identify stable parts of the protein structure. A complex of curcumin and TNF-α binds tightly to certain protein regions that may control how enzymes work. This makes curcumin a good choice for treating inflammation-related back pain (Figure 6).

Normal Mode Analysis (NMA) is used to look at the structure and flexibility of the IL-6 active site when it is combined with curcumin. Figure 7a shows the three-dimensional model of IL-6 attached to curcumin. It shows areas of flexible proteins as colored squares. Different temperature scale colors in the figure depict flexible protein regions against rigid protein areas. Certain areas in the deformability plot (Figure 7b) that show a lot of flexibility point to possible allosteric sites and help figure out regions important for ligand attachment.

Figure 7c displays how the IL-6 protein changes when B-factor analysis is used on both NMA and experimental PDB data. The computer modeling method matches experimental B-factors from the Protein Database to show that it can accurately predict how flexible IL-6 is in its natural state. The eigenvalue distribution chart (Figure 7d) shows how much energy is needed for structural changes. Lower eigenvalues show structural changes that happen more quickly and with less energy when curcumin binds to the receptor protein. A few significant modes dominate the overall motion of IL-6, and the variance plot (Figure 7e) reveals their contribution.

The covariance matrix visualization (Figure 7f) shows how the IL-6 residues are connected by showing how they move in ways that either match or go against each other. This shows the coordinated structural areas. A clear knowledge of IL-6 structural transformations requires an understanding of its dynamic changes when exposed to binding ligands. The elastic network model (Figure 7g) shows how different atomic pairs interact with each other based on the color intensity, which reflects the strength of their bond. The results show that when curcumin binds to IL-6, it stabilizes it. This may help explain how it can help reduce inflammation by controlling the structure of the cytokine in a dynamic way (Figure 7).

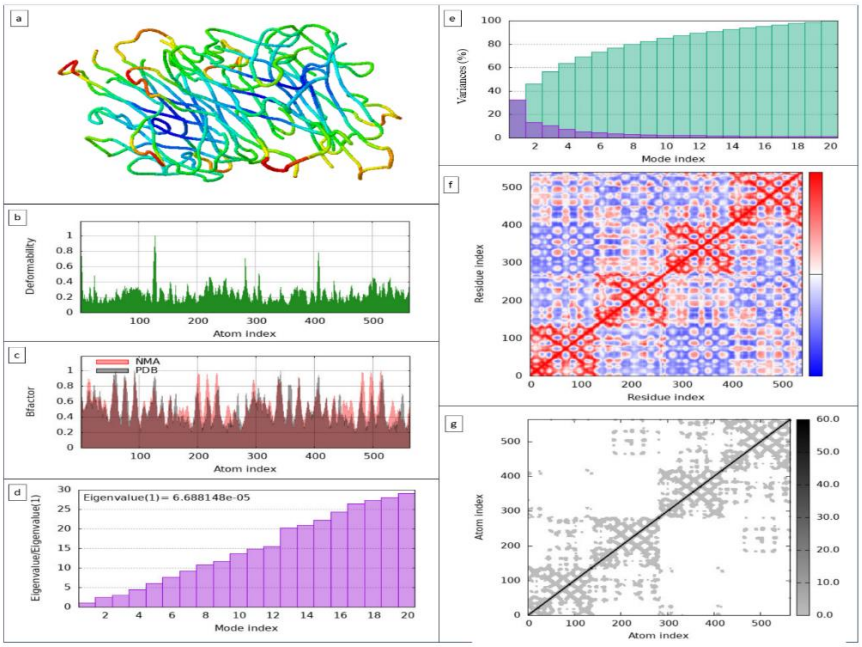

Figure 6: Normal Mode Analysis (NMA) of the TNF-α (PDB ID: 2AZ5) and Curcumin complex. (a) Visualization of the protein-ligand complex with color-coded mobility regions. (b) Deformability plot highlighting flexible regions. (c) B-factor analysis comparing NMA and PDB data. (d) Eigenvalue distribution indicating the energy required for conformational changes. (e) Variance distribution of different normal modes. (f) Covariance matrix showing correlated and anti-correlated residue motions. (g) Elastic network model depicting interaction strength between atomic pairs. These analyses provide insights into the structural stability and dynamic behavior of the TNF-α-Curcumin complex.

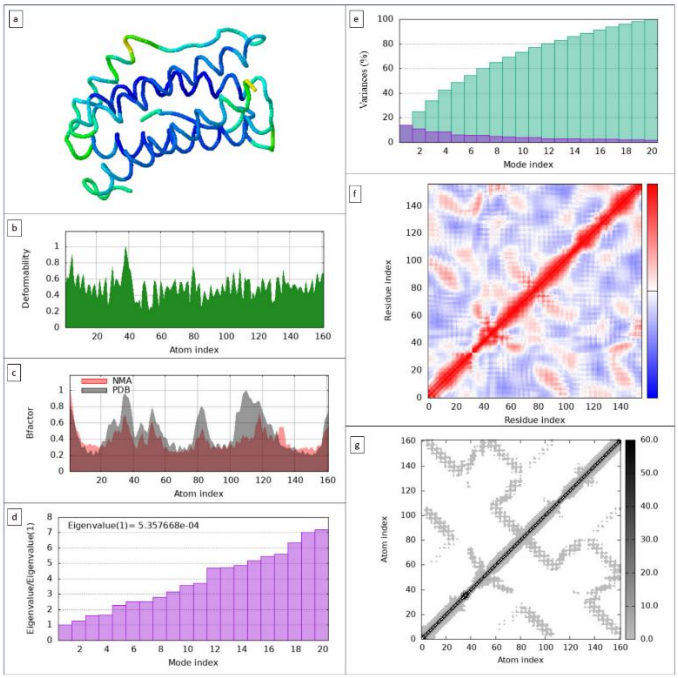

Figure 7: Normal Mode Analysis (NMA) of the Interleukin-6 (IL-6) and Curcumin complex. *(a)* 3D representation of IL-6 bound to Curcumin, with color-coded regions indicating structural flexibility (red: highly flexible, blue: rigid). *(b)* Deformability plot showing residue-specific flexibility. *(c)* B-factor comparison between NMA (red) and PDB (gray), illustrating structural fluctuations. *(d)* Eigenvalue distribution, where lower values indicate easier conformational changes. *(e)* Variance plot showing the contribution of normal modes to overall motion. *(f)* Covariance matrix depicting correlated (red) and anti-correlated (blue) residue motions. *(g)* Elastic network model indicating interaction strengths between atomic pairs, with darker regions representing stronger interactions.

Discussion

This study investigated the pharmacokinetics of two bioactive compounds curcumin and methoxycurcumin found in *Curcuma longa* (turmeric), and their molecular interactions with key proteins involved in LBP, to see if they could help treat low back pain (LBP) by lowering inflammation. Molecular docking analysis shows that curcumin has a strong binding affinity to COX-2 and TNF-α, which are inflammatory mediators. COX-2 functions as an enzyme to produce inflammatory prostaglandins, which contribute to pain development (Gupta *et al.*, 2013). Managing nonsteroidal anti-inflammatory

drugs (NSAIDs) for low back pain (LBP) is mostly about stopping COX-2 from doing its job. However, these drugs are known to have side effects (Day & Graham, 2013) like stomach problems (Sohail *et al.*, 2023) and heart problems (Domper Arnal *et al.*, 2022).

In this study results showed that curcumin and its derivative strongly bound to COX-2. These results are supported from the literature where researchers have found that curcumin and its derivatives strongly bind to COX-2 and have binding energies that are similar to those of common nonsteroidal anti-inflammatory drugs (Lan *et al.*, 2024). Researchers have found that curcumin forms a direct link with TNF-α, a key cytokine in the development of pain and inflammation (Baj & Seth, 2018). The ability of curcumin to reduce TNF-α activity helps explain its potential as an effective agent for managing low back pain.

Numerous favorable molecular and anti-inflammatory properties of curcumin limit its practical medical use because it does not easily enter the body (El-Saadony *et al.*, 2023). According to this study, curcumin exhibits low water solubility, poor digestive system uptake, and rapid biological breakdown, which reduces the effective curcumin levels in blood cells and target locations. These results are in line with the reporting of Liu *et al.* (2016). Curcumin levels in the blood are either too low to be detected or not present at all, even after taking large amounts of this compound by mouth (Anand *et al.*, 2007). There is an alkaloid in black pepper called piperine that works to stop glucuronidation in the liver and intestines. This is one of the most researched ways to make curcumin more bioavailable. According to Chaudhri and Jain (2023), when curcumin was mixed with piperine, it made the drug 2000% more bioavailable in humans, which made it more useful for treatment.

Along with blocking COX-2 and TNF-α activity, curcumin works well as a medicine because it affects more than one inflammatory pathway (Peng *et al.*, 2021). In current study molecular docking analysis indicated that curcumin showed significant molecular interaction with IL-6. It means that curcumin can stop interleukin-6 (IL-6), which is an inflammatory cytokine involved in the pathophysiology of LBP. IL-6 activity is part of the process of chronic pain development. It sets off inflammatory cascades and makes pain receptors more sensitive. As a result of stopping the production of IL-6, curcumin can lower pain and inflammation in people with LBP (Majumdar *et al.*, 2025).

Computational analysis in this researchedshows that curcumin protects nerve tissue and therefore can help tackle LBP symptoms when the condition involves nerve injuries or develops neuropathic pain. According to literature, curcumin boosts the body's antioxidant defenses, lowers the stress caused by harmful cell compounds, and protects against tissue damage (Farzaei *et al.*, 2018). LBP and other types of chronic pain get worse when there is oxidative stress because the inflammatory response hurts and swells nerves (Mosabbir, 2023). The scavenging mechanism of curcumin frees dangerous radicals while decreasing oxidative damage, which allows it to maintain neuron function and stop chronic pain formation. A new study confirms that curcumin raises the production of brain-derived neurotrophic factor (BDNF), an important substance for nerve repair and regeneration (Monroy *et al.*, 2013).

Furthermore, pharmacokinetics analysis showed that curcumin provides protective benefits to neuronal tissue that makes it especially helpful for patients with LBP caused by nerve-derived problems. The study shows that eating fats along with curcumin makes it easier for the body to absorb. This is because turmeric has strong lipid properties that need fat for maximum

bioavailability (Dei Cas & Ghidoni, 2019). Golden Milk, which combines turmeric with milk or coconut oil, functions as an anti-inflammatory remedy in Ayurvedic medicine (Kulkarni *et al.*, 2023). Curcumin's ability to provide neuroprotective and antioxidant benefits makes it an attractive candidate for LBP management since it protects people with nerve inflammation and individuals suffering from oxidative damage.

Conclusion

The research shows that bioactive compounds from *Curcuma longa* can be used to treat low back pain naturally. They do this by interacting with important inflammatory proteins. Molecular docking analysis showed that the compounds bind well with COX-2, TNF-α, and IL-6 proteins, which lets them control pathways that cause inflammation. The ADMET analysis demonstrated good drug properties that support gastrointestinal absorption while showing low toxicity but showing limited central nervous system penetration because of restricted blood-brain barrier passage. The results of the normal mode analysis showed that these compounds are stable and can bind to specific proteins. This supports their use as anti-inflammatory drugs. However, these findings support the use of these compounds in medicine formulation but still, in vitro and in vivo research is required to validate therapeutic safety and efficacy. This work emphasizes the integration of computational methods in drug discovery, therefore enabling the formulation of new plant-based painkillers.

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