

DESIGN, SYNTHESIS, CHARACTERIZATION AND EVALUATION OF INVITRO ANTI OXIDANT ACTIVITY OF RUBROCURCUMIN

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ABSTRACT

Curcumin, a phytochemical with a bright yellow colour, derived from the rhizome of the plant *Curcuma longa*, family Zingiberaceae has drawn a lot of interest because of its many therapeutic uses, which are related to the presence of specific functional groups in its structure. It possesses anti-inflammatory, antibacterial, antirheumatic, immunomodulatory, and anti-tumour properties, according to several research. Despite its promising therapeutic profile, curcumin's poor aqueous solubility, rapid metabolism, and low systemic bioavailability hinder its clinical application. In response to these limitations, structural modification and complexation strategies have been employed to enhance its pharmaceutical potential. One such derivative, rubrocurcumin, a boron-curcumin complex, has emerged as a promising candidate due to its improved chemical stability and enhanced biological activity. The present study evaluated the synthesized compound for its in vitro antioxidant activity and in silico biological potential through molecular docking studies. The "low" hydrolytic stability in specific conditions makes these complexes the candidates for controlled-release carrier systems of curcumin in the human body. By integrating insights from medicinal chemistry, pharmacokinetics, and pharmacodynamics, this research work highlights rubrocurcumin as a valuable scaffold for future drug development within the pharmaceutical sciences.

KEYWORDS: Curcumin, Boron complexes, Drug design, Rubrocurcumin.

INTRODUCTION

The chemical name for curcumin is diferuloylmethane ($C_{21}H_{20}O_6$), which has a molecular mass of 368.37 g/mol. It has a melting point of 183 °C and is a polyphenol compound. Feruloyl groups take the place of

two hydrogen atoms in the structure of curcumin, a beta-diketone. At least two tautomeric forms, enol and keto, are known to exist. Curcumin's enol form is found in alkaline pH media, but its keto form is found in acidic and neutral pH media.

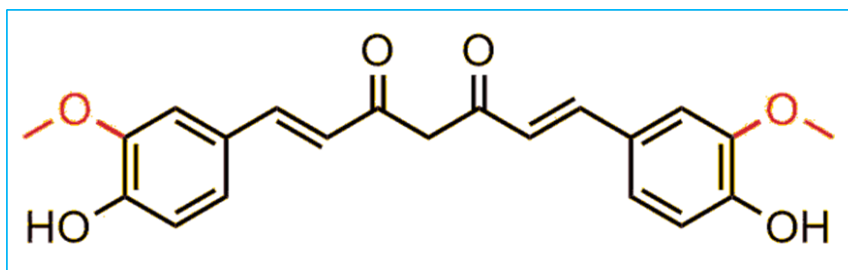


Figure 1: Chemical Structure of Curcumin.

The structural components consist of a seven-carbon linker molecule, an aromatic O-methoxy-phenolic group, an α,β -unsaturated β -diketone moiety. Curcumin has two α,β -unsaturated carbonyl groups that link its aromatic rings. The α,β -unsaturated carbonyl undergoes nucleophilic addition, whereas the diketones readily

deprotonate and create enolates. While the carbon linker moiety contributes hydrophobicity, its phenolic group is responsible for its antioxidant action. Curcumin has been structurally altered to enhance its biological activity because it is naturally hydrophobic. When the pH is raised above 7, the colour of curcumin turns dark red. At

pH values between 2.5 and 7.0, it forms a bright yellow solution.^[1,2]

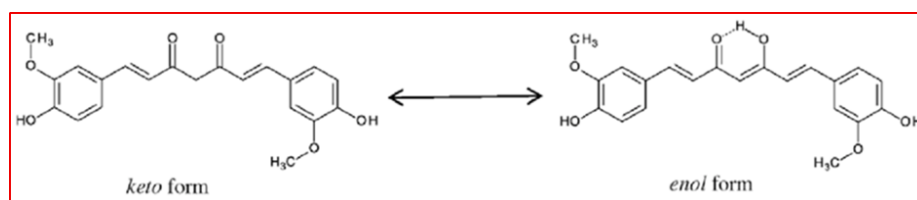


Figure.2. Existence of curcumin in keto-enol tautomeric form.

Numerous pharmacological actions of curcumin were discovered. Curcumin possesses anti-inflammatory, antibacterial, antirheumatic, immunomodulatory, and anti-tumour properties, according to several research. The metallo compounds of curcumin have been shown in recent studies to improve the biochemical activity of curcumin. Metal complexes derived from natural products have become more and more popular as pharmaceuticals in recent decades for use as chemotherapeutic or diagnostic medications. Additionally, metal-curcumin complexes have shown promise in treating a number of chronic illnesses, such as cancer, osteoporosis, arthritis, and neurological conditions including Alzheimer's disease. The modulation of inflammatory mediators, transcription factors, protein kinases, lipid peroxidation, and antioxidant enzymes were linked to these biological activities of metal-curcumin complexes. Furthermore, metal-curcumin complexes have demonstrated utility in radio and biological imaging. Curcumin typically forms metal-curcumin complexes by reacting with various metals through its diketone group. Many metal ions, such

as boron, iron, zinc, cobalt, copper, gallium, chromium, platinum, manganese, nickel, palladium, gold, silver, mercury, aluminium, indium, tin, gadolinium, and lanthanum, are said to be compatible to curcumin's efficient binding.^[3,4]

RUBROCURCUMIN

Spicer & Strickland identified and structurally examined rubrocurcumin in 1952. The Spiro borate ester rubrocurcumin, known as 1,3,2-dioxaborines, [2-[[1,7-bis(4-hydroxy-3-methoxyphenyl)-5-oxohepta-1,3,6-trien-3-yl]oxy]-1,3,2-dioxaborolane-4,5-dione], is created when curcumin, boric acid, and oxalic acid condense. One curcumin and one oxalate ligand tetrahedrally co-ordinate boron to form the neutral molecule known as rubrocurcumin. The molar mass of the compound is $466.21 \text{ g}\cdot\text{mol}^{-1}$, and its chemical formula is $\text{C}_{23}\text{H}_{19}\text{BO}_{10}$. Rubrocurcumin appears as a red solid. Rubrocurcumin is produced when curcumin reacts with borate ions in the presence of oxalic acid.

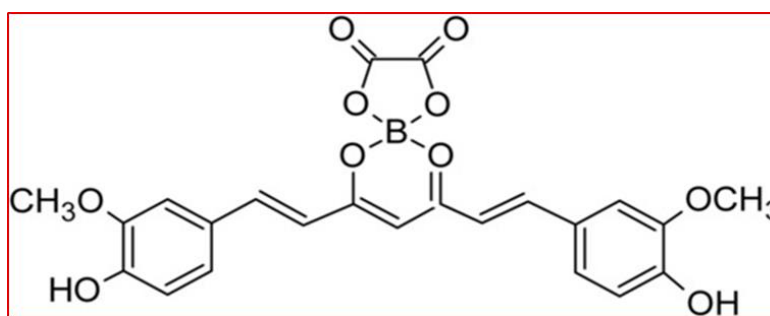


Figure 3. Chemical Structure of Rubrocurcumin.

Compared to curcumin, it has been found to be more thermally stable and also retain their antioxidant properties. It is traditionally employed in the microscale measurement of boron in plant and soil samples. Rubrocurcumin is an intriguing option for selective curcumin delivery because of its capacity to release curcumin under a range of physiological and aqueous conditions.^[5-9]

The present study involved the synthesis of rubrocurcumin, its characterization by UV-Vis spectroscopy, FTIR spectroscopy, TLC, HPLC, proton NMR, Mass spectrum and evaluation of *in vitro* antioxidant activity using the DPPH assay.

MATERIALS AND METHODS

1. Chemical Synthesis of Rubrocurcumin^[10]

Boric acid (62.0 mg, 1.0 mmol) was dissolved in approximately 3 mL of 95% ethanol under stirring (240 rpm) with mild heating (30 °C). Oxalic acid (126.4 mg, 1.0 mmol) was separately dissolved in approximately 3 mL of 95% ethanol. Curcumin (368.6 mg, 1.0 mmol) was dissolved in approximately 62 mL of 95% ethanol under stirring (240 rpm) and heating at 65 °C. The three solutions were combined in a round-bottom flask. The resulting solution exhibited a bright red colour and was heated to reflux overnight. Following reflux, the solution remained bright red and was subsequently transferred to a pre-weighed Petri dish and allowed to evaporate under a fume hood overnight. After complete solvent removal,

the obtained solid appeared red under transmitted light and green under reflected light. The solid was washed with approximately 7 mL of Milli-Q water and 2.5 mL of

95% ethanol to remove residual impurities. The product was then dried overnight prior to further use.



Figure 4: Experimental setup for the chemical synthesis of Rubrocurcumin.

2. Physicochemical Characterization

Appearance/Colour: Red complex.

Molecular Formula: $C_{23}H_{19}BO_{10}$.

Molecular Weight: 466.2 g/mol.

Melting point and solubility of Rubrocurcumin-The compound was found to decompose above 208 °C. Rubrocurcumin is insoluble in water but soluble in organic solvents such as ethanol, methanol and acetone.

3. Thin Layer Chromatography (TLC) analysis of Curcumin and Rubrocurcumin

The mobile phase was prepared by using chloroform and methanol in the ratio of 9: 1 (v/v). The spotted TLC plates were placed inside the saturated TLC chamber containing the mobile phase. The plates were allowed to develop until the solvent front moved to an appropriate height. The plates were then removed from the chamber and air-dried. The spraying reagent was prepared by mixing boric acid, glacial acetic acid, ethanol and distilled water. The developed TLC plates were evenly sprayed with the prepared boric acid spraying reagent. Coloured spots corresponding to curcumin and rubrocurcumin were observed and compared.

4. High Performance Liquid Chromatography (HPLC) analysis of Curcumin and Rubrocurcumin^[11,12]

The quantitative estimation of rubrocurcumin was performed using a reverse-phase high-performance liquid chromatography (RP-HPLC) system. Chromatographic

analysis was carried out and separation of analytes was achieved on a reverse-phase C18 G column. The mobile phase consisted of acetonitrile/methanol and water in a 50: 50 (v/v) ratio, acidified with 0.1% phosphoric acid /2% acetic acid. The mobile phase was filtered through a 0.45 µm membrane filter and degassed prior to use. Isocratic elution was employed throughout the analysis. The flow rate was maintained at 1.0-1.2 mL/min. The detection wavelength was set at 425 nm for the quantification of rubrocurcumin using the PDA detector. The injection volume was 10-20 µL. The column temperature was maintained at 30°C. Under these optimized chromatographic conditions, rubrocurcumin exhibited a retention time of approximately 11.5 minutes in the acetonitrile: acidified water (50: 50, v/v) system. In alternative methanol: acidified water systems (80: 20, v/v), the retention time was observed to be approximately 5.1 minutes.

5. UV-Visible Spectral Analysis of Rubrocurcumin

Spectrophotometric analysis was performed using a double-beam UV-Visible spectrophotometer. A stock solution of the sample (100 µg/mL) was prepared in methanol and subsequently diluted to obtain a working solution of 30 µg/mL. The solution was scanned over a wavelength range of 200–800 nm using methanol as the blank and the spectrum was recorded.

6. Infrared (IR) Spectral Analysis of Rubrocurcumin

The (FTIR) spectral analysis of rubrocurcumin was carried out to identify the characteristic functional groups and to confirm structural features of the synthesized compound. The analysis was performed using a FTIR spectrophotometer. A zinc selenide (ZnSe) ATR crystal was used for sample analysis. A small quantity of rubrocurcumin was placed directly onto the surface of the ATR crystal, ensuring good contact between the sample and the crystal. The FTIR spectrum was recorded in the mid-infrared region over a scan range of 600–4000 cm^{-1} . Each spectrum was obtained by accumulating 30 scans at a spectral resolution of 4 cm^{-1} to improve the signal-to-noise ratio. Background correction was performed prior to sample scanning. The obtained spectrum was analyzed for characteristic absorption bands corresponding to functional groups present in rubrocurcumin.

7. Proton NMR Spectroscopy of Curcumin and Rubrocurcumin

The Proton Nuclear Magnetic Resonance spectroscopy (^1H NMR) spectrum was recorded on a JEOL ECZ-R 500 MHz NMR spectrometer. Approximately 5–50 mg of the sample was dissolved in a deuterated solvent to avoid interference from solvent proton signals and transferred into a glass NMR tube. The tube was inserted into the spectrometer and the magnetic field homogeneity was optimized by shimming, while the instrument was locked to the deuterium signal of the solvent to correct magnetic field drift. The sample was excited with a short radiofrequency pulse at the Larmor frequency, and the emitted signal was recorded as Free Induction Decay (FID).

8. Mass Spectroscopy of Curcumin and Rubrocurcumin

The High-Resolution Mass Spectrometry (HRMS) analysis was performed using a Water Synapt XS mass spectrometer to determine the exact molecular formula of the compound by measuring the mass-to-charge ratio (m/z) with high accuracy (up to four decimal places). The sample was dissolved in volatile LC-MS grade solvents such as acetonitrile, methanol, or water, usually at a dilute concentration, and acids like Formic Acid were sometimes added to improve ionization. After preparation, the sample was ionized using suitable techniques such as Electrospray Ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI), Matrix-Assisted Laser Desorption/Ionization (MALDI), or Electron Ionization (EI) depending on the nature of the analyte. The generated ions were separated according to their m/z values using high-resolution mass analyzers such as Time-of-Flight mass analyzer, Orbitrap mass analyzer, or Fourier Transform Ion Cyclotron Resonance mass spectrometer.

9. *Insilico* molecular docking studies

Molinspiration

Galaxy Visualizer is a web-based tool that facilitates the generation of three-dimensional molecular structures from SMILES notation using the Molinspiration Galaxy 3D generator.^[13]

Molecular docking studies

The computational analysis was performed using a computer equipped with an Intel i7 processor and software applications including BIOVIA Discovery Studio Visualizer and MZ Dock. Furthermore, online resources such as the RCSB Protein Data Bank, and the PubChem database were utilized in the study.

MZDock

MZ Dock is a graphical user interface (GUI)-based docking pipeline developed for the Windows operating system to streamline molecular docking procedures and improve reproducibility, particularly for novice users. In addition to batch scripting and Python integration, MZ Dock offers several functional features, including configuration of binding sites based on co-crystallized ligands, enantiomer generation from SMILES input, energy minimization using multiple force fields (MMFF94, MMFF94s, UFF, GAFF, and Ghemical), retention of selected ions and cofactors, compatibility with multiple input file formats (SMILES, PDB, SDF, Mol2, Mol), side-chain flexibility for selected binding site residues, and automated generation of reports and images for interactive visualization.^[14]

BIOVIA Discovery Studio

BIOVIA Discovery Studio is a comprehensive software platform used for visualization and analysis of molecular docking results. It provides analytical tools to evaluate ligand–target complexes, including calculations of binding free energy, binding affinity, and related molecular interactions. The software also features an intuitive interface that enables customization of molecular representations and animations, facilitating the production of high-quality graphical outputs.^[15]

Protein Data Bank

The RCSB Protein Data Bank (PDB; <http://www.rcsb.org/pdb/>) serves as the global archive for three-dimensional structural data of biological macromolecules.^[16]

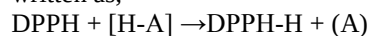
PubChem

PubChem (<https://pubchem.ncbi.nlm.nih.gov>) is an open-access repository that provides comprehensive information on chemical substances and their biological activities. Established in 2004 as part of the Molecular Libraries Roadmap Initiative of the US National Institutes of Health (NIH), PubChem has evolved into a major chemical information resource supporting research in cheminformatics, chemical biology, medicinal chemistry, and drug discovery.^[17]

10. In-vitro Antioxidant Activity by DPPH Assay^[18-29]

The radical scavenging activity of different extracts was determined by using DPPH assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm.

Principle: 1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with pink colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,



Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential

of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Procedure

Different concentrations of sample such as 12.5µg/mL-200µg/mL from stock solution of 10 mg/mL were made up to a final volume of 20µl with DMSO were taken and 1.48ml DPPH (0.1mM) solution was added. A control without the test compound, but an equivalent amount of distilled water was taken. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control.

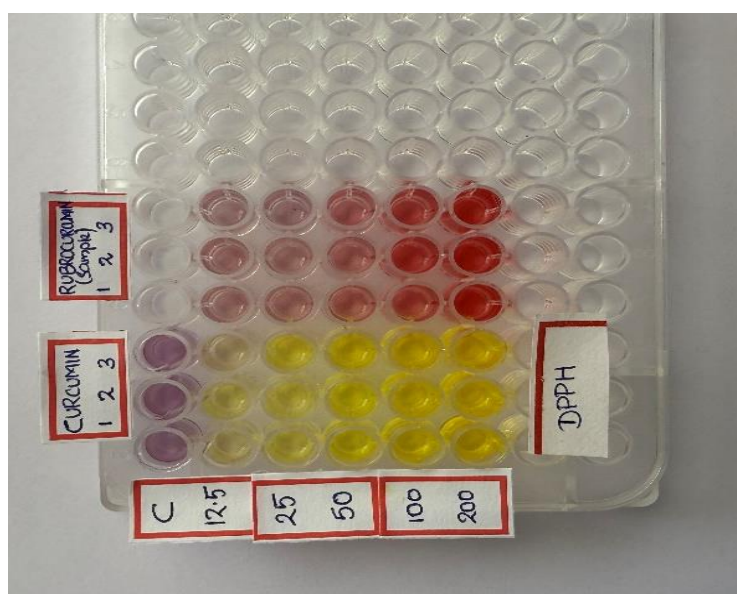


Figure 5: Concentrations of Curcumin and Rubrocurcumin for the DPPH assay.

11. Comparison of Antioxidant Activity of Curcumin and Rubrocurcumin

The antioxidant activity of curcumin and rubrocurcumin was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Stock solutions of both compounds were prepared and diluted to obtain a series of concentrations. An aliquot of each concentration was mixed with a freshly prepared DPPH solution and incubated in the dark at room temperature for a specified period. Following incubation, the decrease in absorbance was measured spectrophotometrically at 517 nm. The percentage of DPPH radical scavenging activity was calculated for each concentration using a standard formula. A concentration-response curve was plotted, and the IC₅₀ value, defined as the concentration required to scavenge 50% of DPPH radicals, was calculated using ED50 Plus V1.0 software. The antioxidant activities of curcumin and rubrocurcumin were compared based on their respective IC₅₀ values,

with lower IC₅₀ values indicating greater antioxidant potential.

12. Statistical Analysis of Experimental data

DPPH assay experiments for curcumin and rubrocurcumin were performed in triplicate, and the results were expressed as mean ± SD. The percentage of inhibition (DPPH radical scavenging activity) was calculated using the equation:

$$\text{Percentage of inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

RESULTS AND DISCUSSION

CHEMICAL SYNTHESIS OF RUBROCURCUMIN

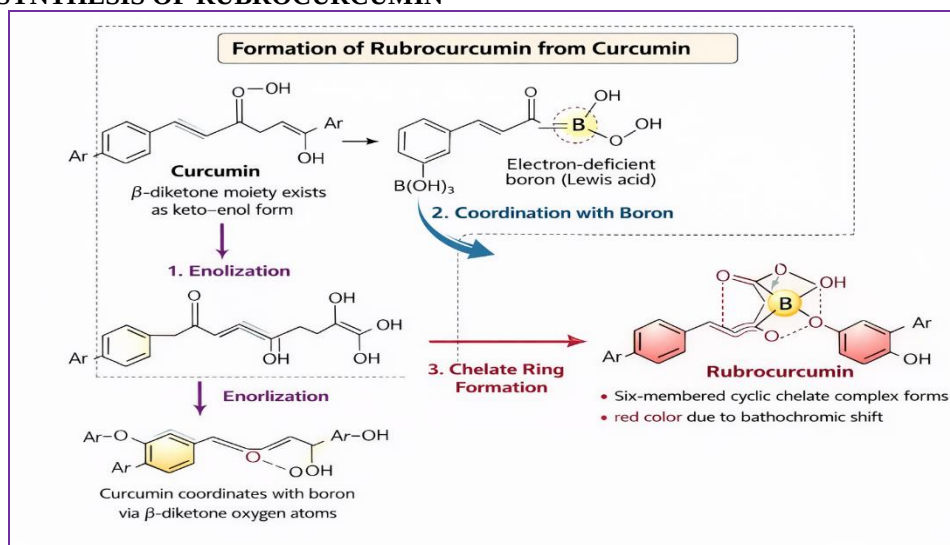


Figure 6. Formation of Rubrocurcumin.

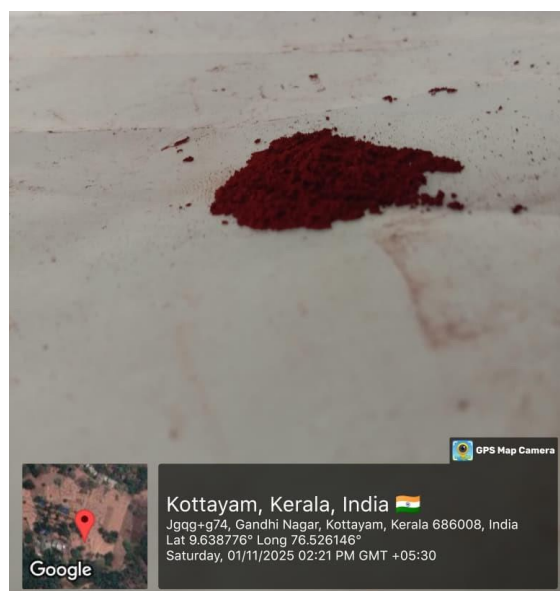


Figure 7. Synthesized Rubrocurcumin.

THIN LAYER CHROMATOGRAPHY

Mobile phase : Chloroform : Methanol = 9: 1

Distance travelled by the solvent front from origin = 5.8 cm

Distance travelled by the Curcumin = 4 cm

Distance travelled by the Rubrocurcumin = 3.2 cm

Table 1: TLC profile of compounds.

COMPOUND	COLOUR	OBSERVED R_f VALUE
Curcumin	Yellow-Orange	0.68
Rubrocurcumin	Red	0.55

Curcumin produced a yellow-orange spot with an R_f value of 0.68, while rubrocurcumin exhibited a red spot with a lower R_f value of 0.55 under identical chromatographic conditions. The difference in R_f values suggests variation in polarity and interaction with the stationary phase, thereby indicating successful

conversion of curcumin to rubrocurcumin. The distinct color change further supports the formation of the synthesized complex.



Figure 8: TLC of Rubrocurcumin(red) and Curcumin(orange) showing distinct spots.

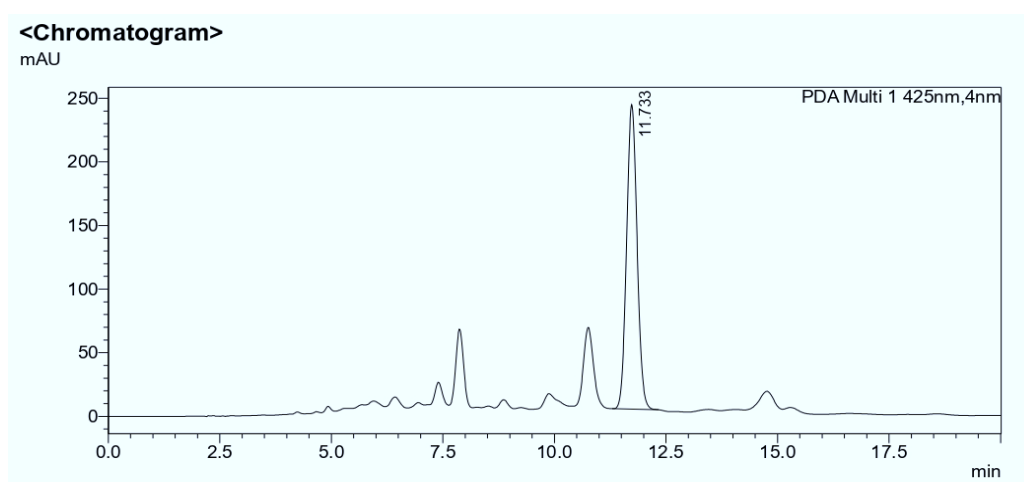


Figure 9: HPLC chromatogram of synthesized Rubrocurcumin.

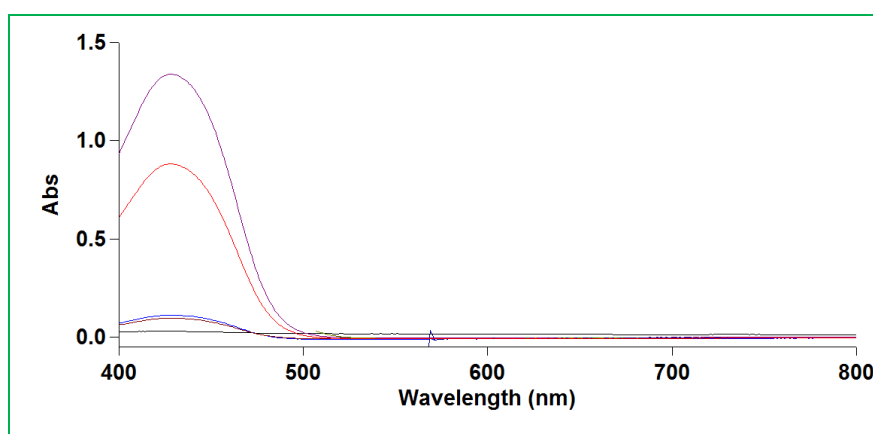


Figure 10: UV-Visible spectrum of Curcumin.

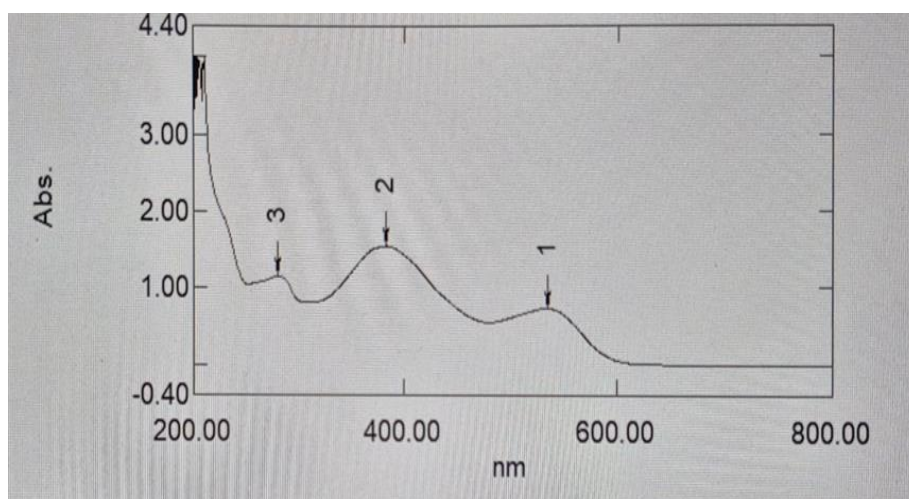


Figure 11: UV – Visible spectrum of synthesized Rubrocurcumin.

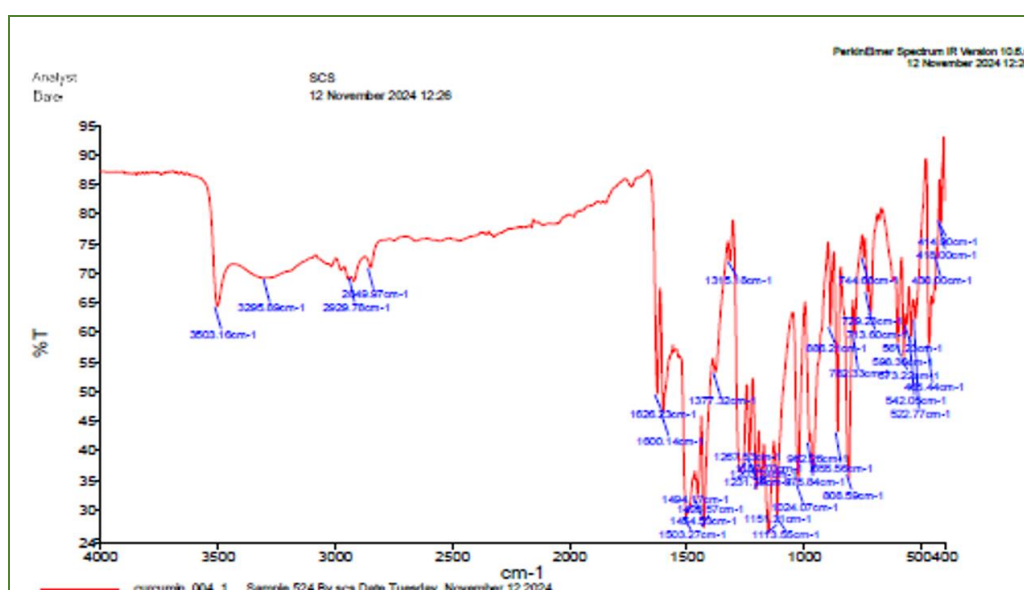


Figure 12: FTIR spectrum of Curcumin.

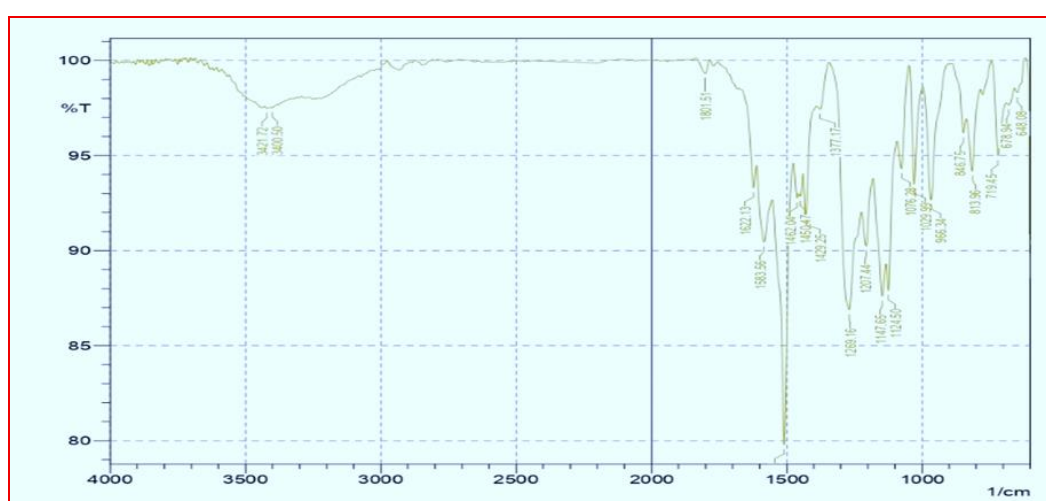


Figure 13: FTIR spectrum of synthesized Rubrocurcumin.

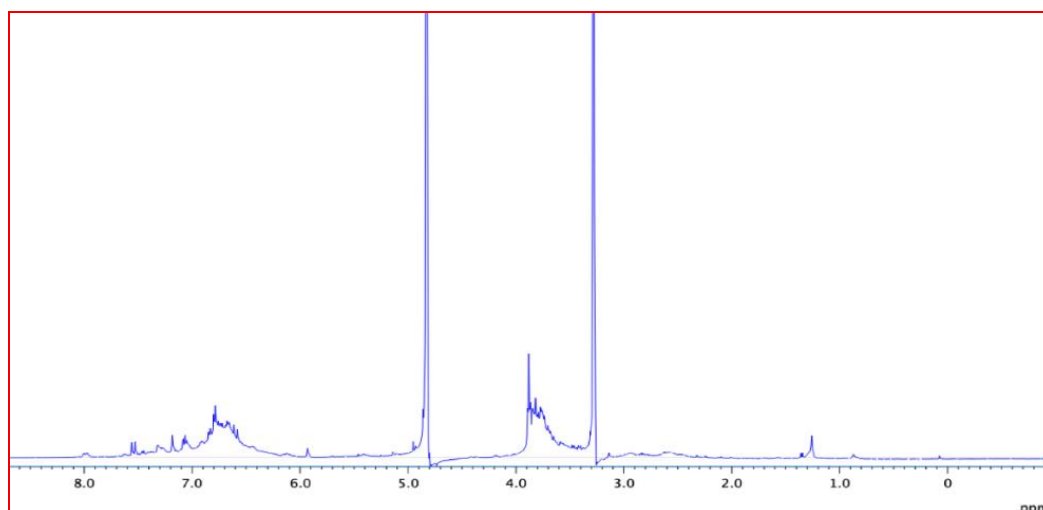


Figure 14: ^1H NMR spectrum of synthesized Rubrocurcumin.

The major spectral evidence for the formation of rubrocurcumin from curcumin is the disappearance of the enolic proton signal (~ 16 ppm) and slight chemical shift

changes in aromatic and olefinic protons, confirming the boron–curcumin chelate formation.

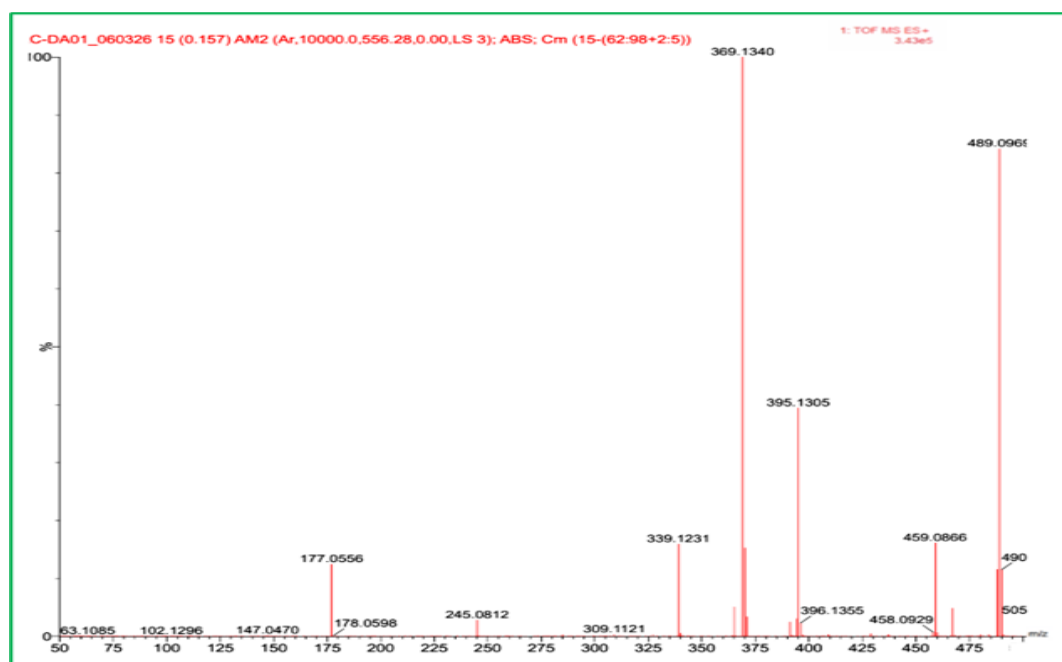


Figure 15. Mass spectrum of synthesized Rubrocurcumin.

The ESI-TOF mass spectrum of the compound shows a prominent molecular ion peak at m/z 489.09, corresponding to the protonated molecular ion $[\text{M}+\text{H}]^+$, indicating a molecular weight of approximately 488 Da. The mass spectrum of rubrocurcumin differs significantly from that of curcumin, mainly by the increase in molecular ion peak from m/z 369 to m/z ~ 489 and the appearance of additional higher-mass fragment ions such as m/z 395, 459. The peak at m/z 369, which corresponds to the curcumin fragment, indicating breakdown of the complex. These changes confirm the

formation of the boron–curcumin complex (rubrocurcumin).

The formation of rubrocurcumin from curcumin involves boron chelation at the β -diketone moiety. The following spectroscopic changes support the proposed coordination mechanism.

Table 2": Comparison of Spectroscopic Changes Supporting the Mechanism of Rubrocurcumin Formation.		
Technique	Observed Change	Mechanistic Interpretation
UV-Visible Spectroscopy	Bathochromic shift from ~425 nm to ~535 nm	Increased π -electron delocalization due to boron chelation, reducing the HOMO-LUMO energy gap.
IR Spectroscopy	Shift/alteration in carbonyl (C=O) stretching region and changes in C-O bands	Coordination of β -diketone oxygen atoms with boron forming a six-membered chelate ring.
^1H NMR Spectroscopy	Disappearance of the characteristic enolic OH proton (~16 ppm) present in curcumin	The enolic proton participates in boron chelation, confirming formation of the boron-curcumin complex.
Mass Spectrometry (ESI-TOF)	Molecular ion peak shift from m/z 369 ($[\text{M}+\text{H}]^+$) in curcumin to m/z ~489 ($[\text{M}+\text{H}]^+$)	Increase in molecular mass due to formation of the rubrocurcumin complex with boron.
HPLC Analysis	Retention time shift from ~6.98 min to 11.733 min	Increased hydrophobicity and structural modification after complex formation.
Physical Observation	Color change from yellow to deep red	Extended conjugation in the boron complex leading to absorption in the green region (bathochromic shift).

Collectively, the spectroscopic evidence confirms the successful boron coordination with the β -diketone moiety of curcumin, resulting in the formation of a stable rubrocurcumin chelate complex.

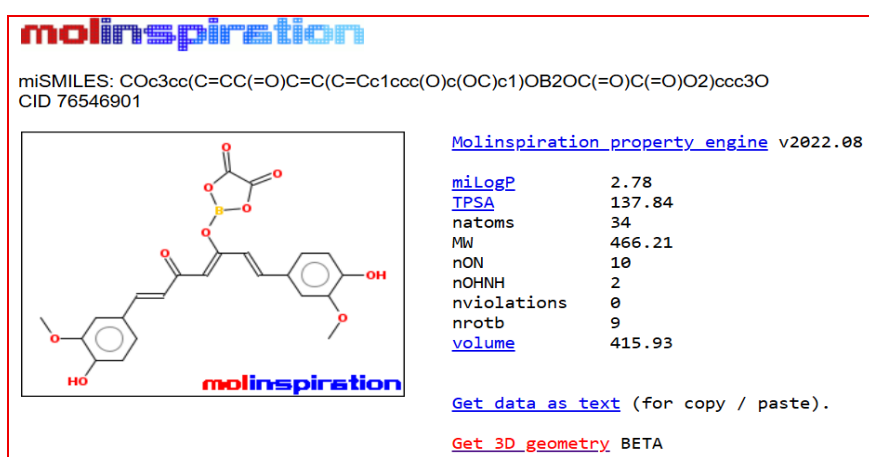


Figure 16: Molinspiration property of Rubrocurcumin.

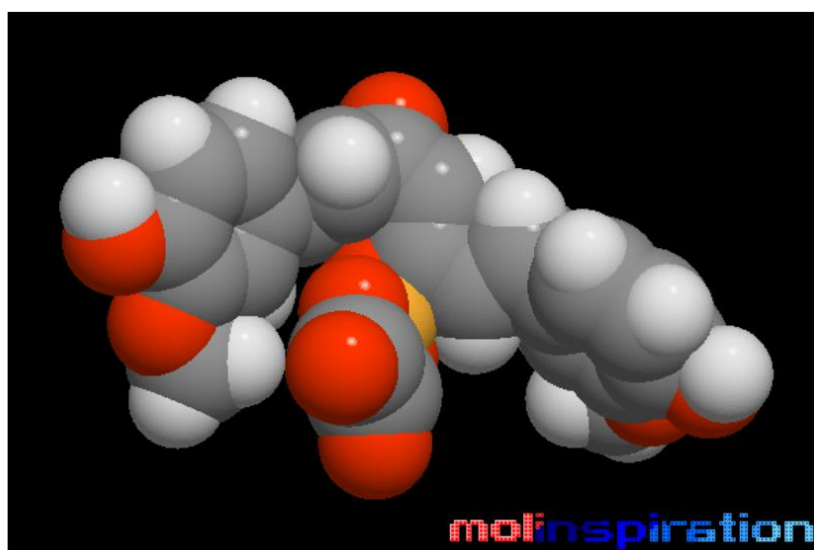


Figure 17: 3D Geometry of Rubrocurcumin.

DOCKING RESULTS

Table 3: Overview of Compounds Structure and Activities.

COMPOUND	STRUCTURE	ACTIVITY POTENTIAL
Curcumin	Polyphenolic diarylheptanoid containing two aromatic rings connected by a conjugated β -diketone moiety with phenolic hydroxyl groups	Known anti-oxidant anti-inflammatory, anti-cancer, and anti-microbial activity.
Rubrocurcumin	Curcumin-boron complex in which curcumin coordinates to boron from boric acid through its β -diketone moiety, while oxalic acid acts as a stabilizing ligand to complete the chelation.	Reported to exhibit antioxidant activity with altered molecular interaction characteristics compared to curcumin and exhibits improved chemical stability compared to curcumin.

RESULTS OF DOCKING STUDIES & TOXICITY PROFILE

Table 4: Molecular docking results of compounds against the selected Anti-Cancer target receptor protein.

LIGAND	TARGET RECEPTOR PROTEIN (PDB ID)	BINDING ENERGY (kcal/mol)
Curcumin	6JQR	-7.7
Rubrocurcumin		-8.1
Gilteritinib		-8.2



Figure 18: Target Protein Structure for Anti Cancer Activity Analysis.

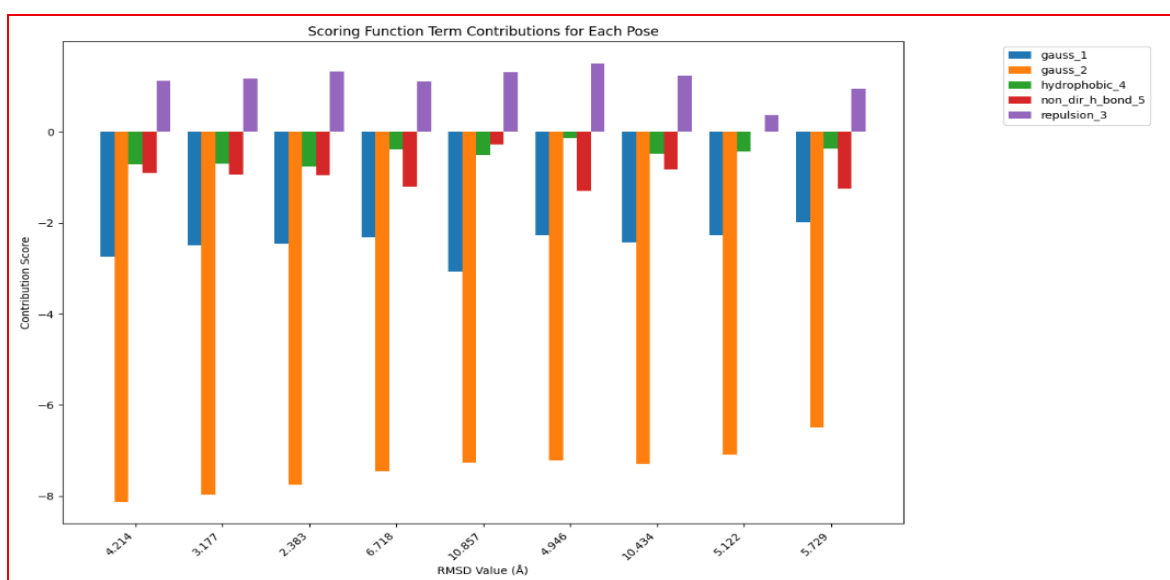


Figure 19: Scoring Function Contributions for Anti Cancer Ligand Docking Poses.

Table 5: Molecular docking results of compounds against the selected Anti-Alzheimers target receptor protein.

LIGAND	TARGET RECEPTOR PROTEIN (PDB ID)	BINDING ENERGY (kcal/mol)
Curcumin	1EVE	-10.7
Rubrocurcumin		-10.9
Donepezil		-11.4



Figure 20: Target Protein Structure for Anti Alzheimer's Activity Analysis.

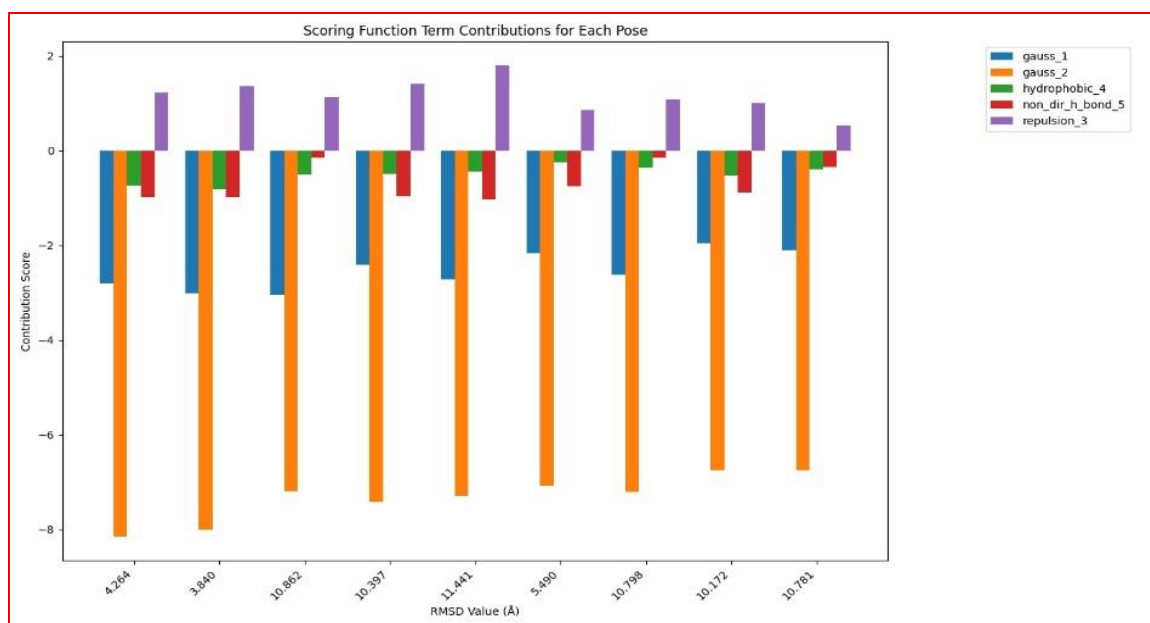


Figure 21. Scoring Function Contributions for Anti Alzheimer's Ligand Docking Poses.

Table 6: Molecular docking results of compounds against the selected Anti-Inflammatory target receptor protein.

LIGAND	TARGET RECEPTOR PROTEIN (PDB ID)	BINDING ENERGY (kcal/mol)
Curcumin	5KIR	-8.3
Rubrocurcumin		-8.8
Rofecoxib		-10.2

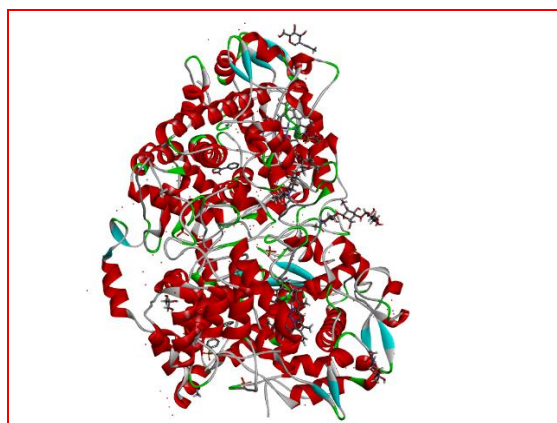


Figure 22: Target Protein Structure for Anti Inflammatory Activity Analysis.

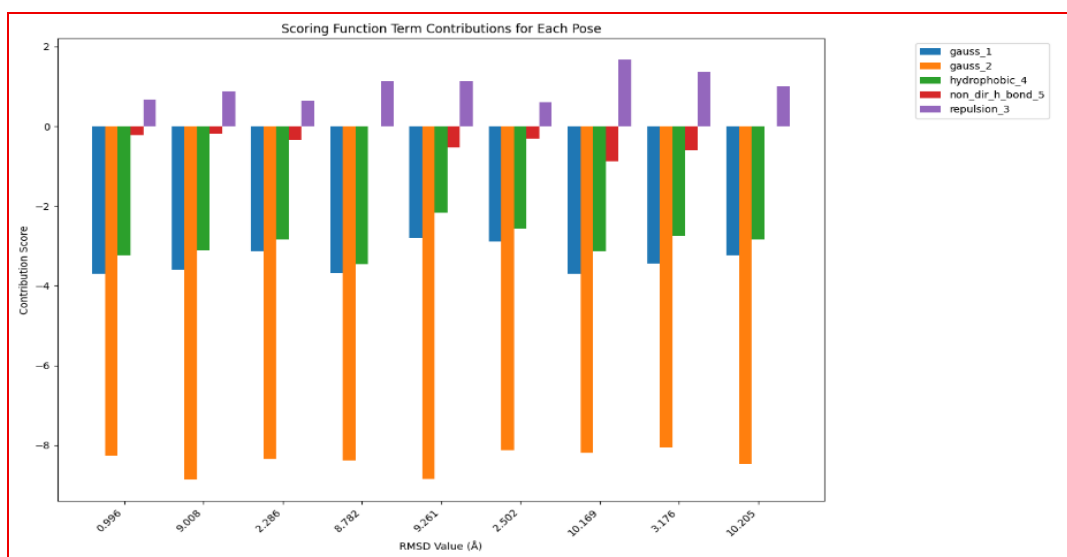


Figure.23: Scoring Function Contributions for Antiinflammatory Ligand Docking Poses.

Table 7: Molecular docking results of compounds against the selected direct anti oxidant target receptor protein.

LIGAND	TARGET RECEPTOR PROTEIN (PDB ID)	BINDING ENERGY (kcal/mol)
Curcumin	5LOX	-4.7
Rubrocurcumin		-3.2
Ascorbic acid		-4.2

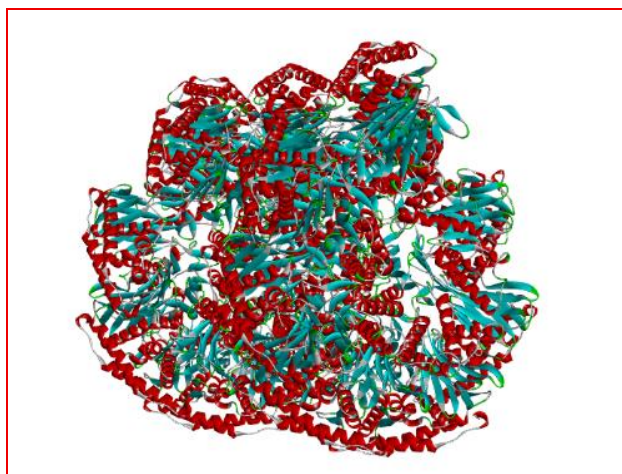


Figure.24: Target Protein Structure for Antioxidant Activity Analysis.

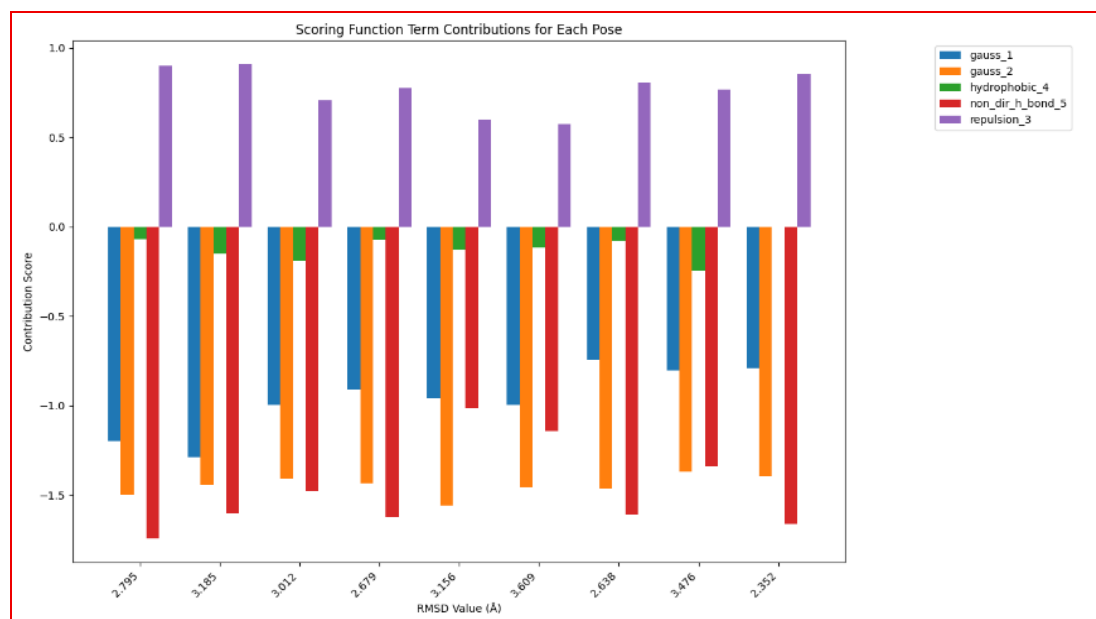


Figure.25: Scoring Function Contributions for Antioxidant Ligand Docking Poses.

Molecular docking studies were performed to evaluate the interaction potential of curcumin and rubrocurcumin with selected target proteins associated with anti-cancer (PDB ID: 6JQR), anti-Alzheimer's (PDB ID: 1EVE), and anti-inflammatory (PDB ID: 5KIR) activities, and the results were compared with standard reference drugs. In all three studies, rubrocurcumin demonstrated comparable or slightly improved binding affinity relative to curcumin, with binding energies of -8.1 kcal/mol (anti-cancer), -10.9 kcal/mol (anti-Alzheimer's), and -8.8 kcal/mol (anti-inflammatory). The reference drugs gilteritinib, donepezil, and rofecoxib exhibited stronger binding interactions, as expected. Hence, the docking results provide computational support for the potential biological relevance of rubrocurcumin; however, as molecular docking is a predictive tool, further experimental validation through *in vitro* and *in vivo* studies is required to confirm the pharmacological activity.

But for the direct anti-oxidant activity docking among the compounds, curcumin demonstrated the strongest binding affinity with a binding energy of -4.7 kcal/mol, followed by the reference antioxidant ascorbic acid with -4.2 kcal/mol, while rubrocurcumin showed comparatively lower binding affinity with a binding energy of -3.2 kcal/mol. The overall binding affinity trend observed was curcumin > ascorbic acid > rubrocurcumin, suggesting that although rubrocurcumin interacts with the antioxidant target, its predicted binding strength is weaker than that of curcumin and the standard ligand, while still supporting its potential antioxidant relevance.

Toxicity prediction studies showed that curcumin shows broader predicted toxicity interactions, particularly involving drug-metabolizing enzymes and cellular stress pathways where as Rubrocurcumin exhibits a simpler and generally safer predicted toxicity profile, with fewer active endpoints.

TOXICITY PROFILE OF CURCUMIN Vs RUBROCURCUMIN

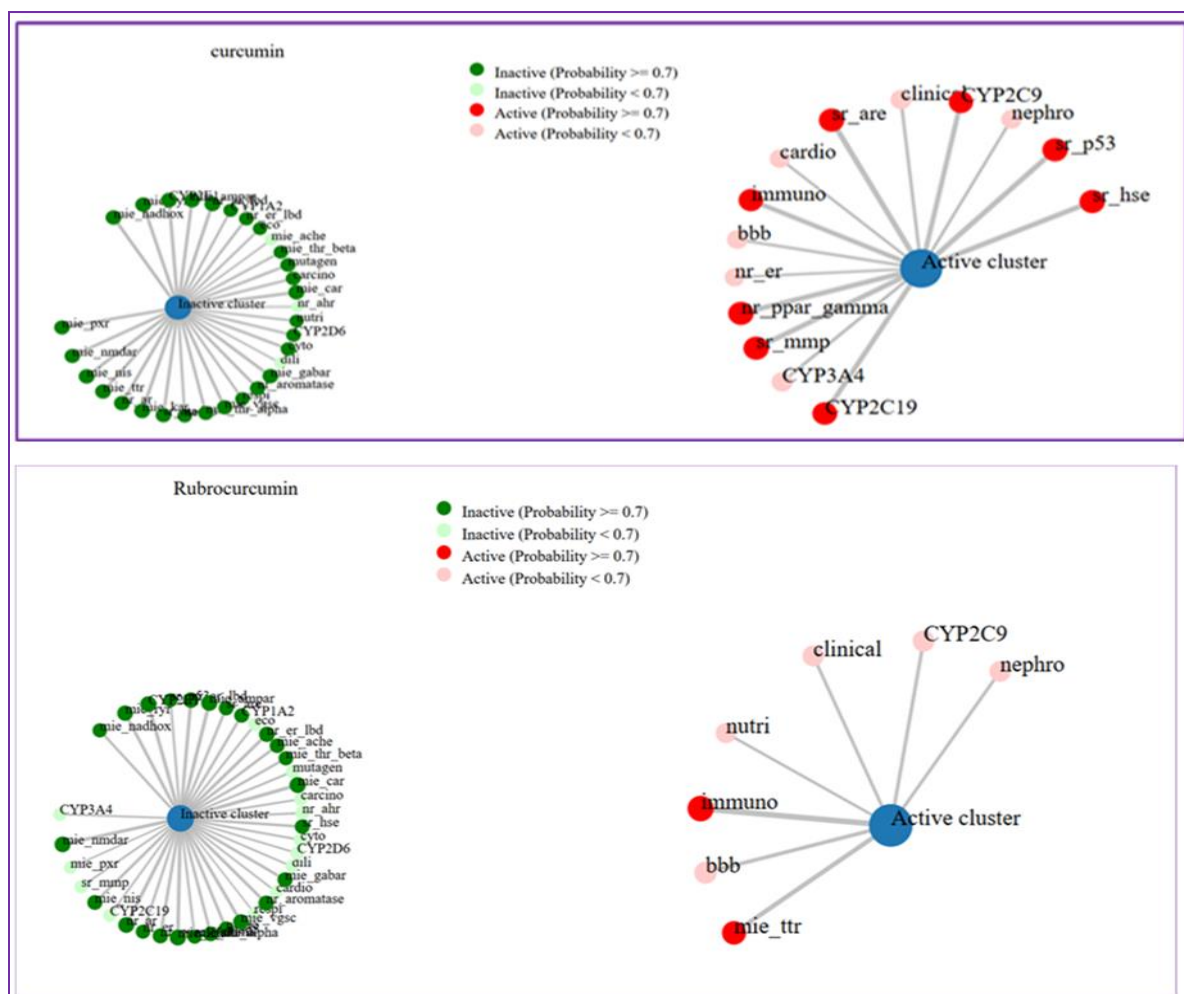


Figure 26: Prediction of Toxicity of Curcumin Vs Rubrocurcumin.

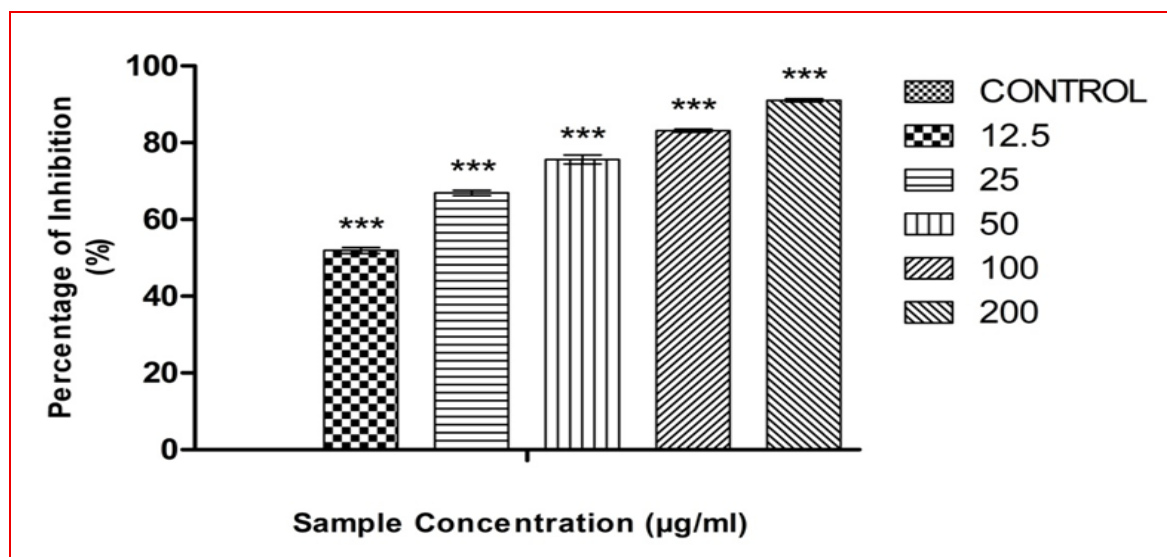


Figure 27: Graphical representation of DPPH Radical scavenging assay in Curcumin.

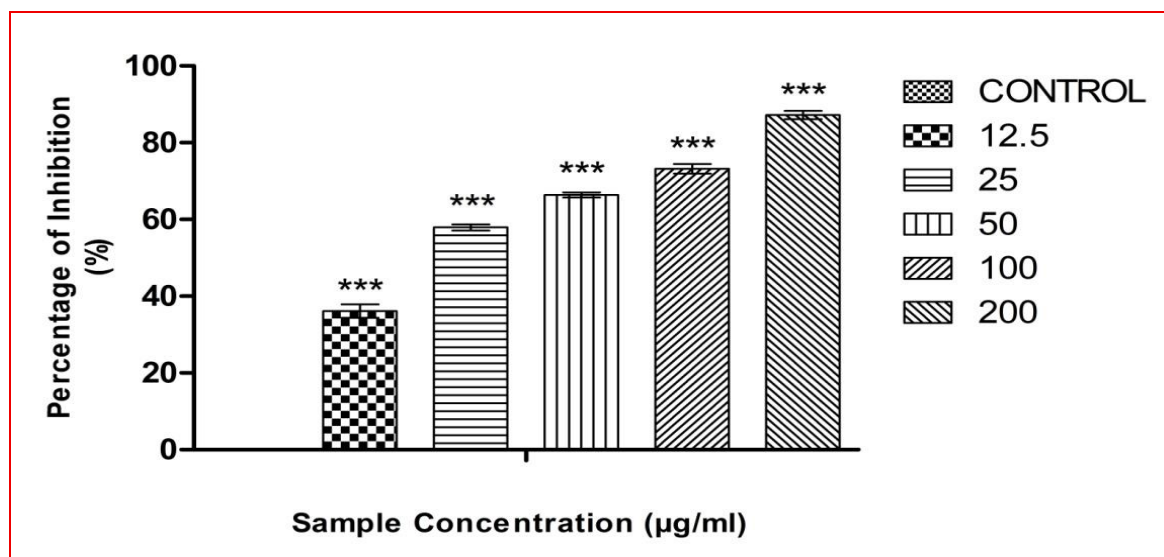


Figure.28: Graphical representation of DPPH Radical scavenging assay in Rubrocurcumin.

Table 8: Statistical Analysis of DPPH Radical scavenging assay results.

STANDARD: CURCUMIN									
	OD1	OD2	OD3	% Inhibition 1	% Inhibition 2	% Inhibition 3	Average	Std.dev	Std.error
Control	0.498	0.516	0.535	0	0	0	0	0	0
12.5	0.241	0.254	0.249	51.6064	50.7752	53.4579	51.9465	1.37333	0.79289
25	0.17	0.172	0.17	65.8635	66.6667	68.2243	66.9181	1.20034	0.69302
50	0.132	0.125	0.12	73.494	75.7752	77.5701	75.6131	2.04289	1.17946
100	0.088	0.084	0.089	82.3293	83.7209	83.3645	83.1382	0.72287	0.41735
200	0.041	0.047	0.051	91.7671	90.8915	90.4673	91.0419	0.66283	0.38268
SAMPLE: RUBROCURCUMIN									
	OD1	OD2	OD3	% Inhibition 1	% Inhibition 2	% Inhibition 3	Average	Std.dev	Std. error
Control	0.498	0.516	0.535	0	0	0	0	0	0
12.5	0.319	0.345	0.325	35.9438	33.1395	39.2523	36.1119	3.05987	1.76661
25	0.203	0.225	0.224	59.2369	56.3953	58.1308	57.921	1.43237	0.82698
50	0.168	0.167	0.186	66.2651	67.6357	65.2336	66.3781	1.20499	0.6957
100	0.142	0.142	0.13	71.4859	72.4806	75.7009	73.2225	2.20325	1.27205
200	0.069	0.055	0.074	86.1446	89.3411	86.1682	87.218	1.83872	1.06158

SUMMARY AND CONCLUSION

Curcumin undergoes keto–enol tautomerism, enabling the β -diketone moiety to act as a bidentate ligand. Upon reaction with boron species, the enolic oxygen and adjacent carbonyl oxygen coordinate to the electron-deficient boron atom, forming a stable six-membered chelate ring. This coordination enhances π -electron delocalization across the conjugated system, resulting in a significant bathochromic shift in the UV–Visible spectrum, the characteristic red colour of rubrocurcumin.

The analysis of the combined spectroscopic and analytical data confirm the formation of the rubrocurcumin complex. The bathochromic shift in the UV–Visible spectrum, disappearance of the enolic proton signal in the ^1H NMR spectrum, and changes in the carbonyl stretching region of the IR spectrum indicate

boron coordination with the β -diketone moiety of curcumin. The increase in the molecular ion peak from m/z 369 to approximately m/z 489 further confirms the formation of a higher molecular weight boron–curcumin complex. In addition, the shift in HPLC retention time (11.733 min) and the visible colour change from yellow to red support the structural modification. These findings collectively validate the successful formation and characterization of rubrocurcumin.

Rubrocurcumin does not exhibit significant increase in *in vitro* antioxidant activity which is primarily due to structural modification caused by boron complex formation, which may reduce the availability of free phenolic hydroxyl groups essential for hydrogen donation. Additionally, altered electronic distribution and steric hindrance may impair radical scavenging

efficiency. Rubrocurcumin is non toxic and found to have significant biological activities similar to curcumin. The oral intake of rubrocurcumin is suggestive, especially in presence of oily food materials in which it is soluble as well as more stable. It undergoes hydrolysis in water-mediated, particularly basic, conditions to release boric acid, oxalic acid and free curcumin, the active drug. Hydrolysis is significantly catalyzed by OH⁻ ions, rubrocurcumin complexes are more stable at lower (acidic) pH and decompose faster in neutral or alkaline conditions. The constant curcumin concentration in the stomach may help with longer absorption of curcumin to bloodstream.

However, the promising *in silico* docking results suggest that the compound may exert various biological effects through enzyme inhibition and pathway modulation rather than direct free radical scavenging. Therefore, although rubrocurcumin does not demonstrate the significant antioxidant activity *in vitro*, computational studies highlight its potential as a lead molecule for further development in anti-inflammatory, anticancer, and anti-Alzheimer's drug research. The low predicted toxicity, highlights its potential as a promising candidates for future drug development.

FUTURE PROSPECTS

The next phase of the research should involve enzyme inhibition assays, cell line studies, *in vivo* pharmacological evaluation and ADME to evaluate the efficacy, pharmacokinetics, bioavailability, and safety of rubrocurcumin in physiological environments to validate the computational predictions and establish its therapeutic relevance. Ultimately, with adequate preclinical data, the promising candidate rubrocurcumin can move forward to clinical assessments, aiding in the development of a controlled-release carrier systems of curcumin in the human body.

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