

RESEARCH ARTICLE



Phytochemical Analysis, Antioxidant and Anti-ulcer Potential of *Gossypium barbadense* L. Leaf Extract (GBLE): *In Vitro* and *In Silico* Approaches

Hannah Ireoluwa Alabi^{1*}, Olu Israel Oyewole¹ and Julianah Bunmi Adetunji¹

¹Department of Biochemistry, Osun State University, Osogbo, Nigeria

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Abstract: *Gossypium barbadense* L. has traditionally been used to manage various ailments. This study evaluated the phytochemical composition, antioxidant potential, and anti-ulcer potential of *G. barbadense* leaf extract (GBLE) using *in vitro* and *in silico* approaches. Qualitative and quantitative phytochemical analyses were performed, while antioxidant activity was assessed using H₂O₂, NO, DPPH, and ABTS assays. GC-MS was used to identify bioactive compounds, followed by molecular docking with PyRx/AutoDock against selected ulcer-related targets (IPSO, NF-κB, and KEAP-1). Phytochemical screening revealed xanthoproteins, tannins, triterpenes, glycosides, saponins, and flavonoids. GBLE showed notable antioxidant activity, while GC-MS identified several bioactive compounds, including methyl stearate, dodecanoic acid methyl ester, tetra-cosane, α-copaene, heneicosane, methyl tetradecanoate, heptadecane, hexadecanoic acid methyl ester, humulene, methyl 9-octadecenoate, β-caryophyllene, and eicosane. Molecular docking revealed favourable binding affinities ranging from -7.0 to -3.4 kcal/mol, suggesting stable ligand-protein interactions. Overall, the findings support the antioxidant and anti-ulcer potentials of *G. barbadense* leaves and suggest that their bioactive constituents may contribute to these effects.

Keywords: Scavenging assays, binding affinities, antioxidants and Ulcer pathogenesis

1. INTRODUCTION

The need to substitute certain medications owing to their adverse effects underscores the universal role of medicinal plants in health maintenance, disease prevention and treatment. Since antiquity, plant therapy has been widely used to manage diseases, including acute and recurrent gastrointestinal disorders such as inflammatory bowel disease and ulcers (Kuna *et al.*, 2019). This reliance is due to the healing potential of medicinal plants, which contain diverse secondary metabolites, including terpenoids, saponins, flavonoids, glycosides, tannins, and alkaloids, which are widely distributed across various plant parts as defensive agents against pathogens. Many of these compounds have shown significant therapeutic benefits in humans, thus attributing their multiple pharmacological actions (Kim *et al.*, 2018). Despite these promising benefits, their applications remain limited due to insufficient scientific evidence, limited pleiotropy, potential toxicity, and standardisation issues. Targeting specificity and efficacy, ensuring safety, and minimising the use of animals in *in vivo* studies are essential steps in optimising the use of medicinal plants. These objectives can be achieved through phytochemical screening and *in silico* analyses to identify and quantify bioactive compounds and to predict their mechanisms of action, toxicity, and efficacy prior to *in vivo* investigations.

*Correspondence should be addressed to Hannah Ireoluwa Alabi, Department of Biochemistry, Osun State University, Osogbo, Nigeria; Tel: +2348039244275; E-mail: hannah.alabi@uniosun.edu.ng

Gastric ulcer, a widespread gastrointestinal disorder, is among the conditions traditionally managed with medicinal plants. It is a type of peptic ulcer characterised by open, painful lesions in the gastric mucosa that often lead to bleeding and increased susceptibility to infection, making it a significant global health concern. Factors contributing to gastric ulcer include the use of Non-Steroidal Anti-inflammatory Drugs (NSAIDs), alcohol consumption, stress, *Helicobacter pylori* infection, and excessive intake of irritant foods such as spicy meals, carbonated beverages, and coffee (Shahzad *et al.*, 2024). Its pathophysiology involves an imbalance between mucosal defence mechanisms and aggressive factors (e.g. gastric acid and pepsin). In addition, gastric inflammation, oxidative stress and impaired antioxidant defences contribute to disease progression. The dysregulation of KEAP-1 and NF- κ B signalling pathways, followed by gastric mucosal injury, further exacerbates ulcer pathogenesis, suggesting that modulation of these pathways may provide therapeutic benefit.

Gossypium barbadense (cotton plant) is widely used in Ayurvedic medicine for conditions such as wound healing, haemorrhoids, haemorrhage control, bronchitis, fungal infections and rheumatism (Ade-Ademilua and Okpoma, 2018). It is a perennial shrub indigenous to South America and is widely cultivated in Senegal and Nigeria. Various parts, such as seeds, leaves, bark, and roots, are used in traditional medicine for their therapeutic potential. The species has been reported to exhibit anticancer, anti-ulcer, antihypertensive, anti-inflammatory, antimalarial, antioxidant, and antimicrobial activities (Morales-Aranibar *et al.*, 2023; Balkrishnan *et al.*, 2023; Olanrewaju *et al.*, 2025). Among all of its traditional applications, wound healing is most prominent, possibly due to gossypol and related compounds identified in the leaf essential oil (Sabiou *et al.*, 2017). However, comprehensive scientific validation of its pharmacological effects, particularly regarding anti-ulcer activity and wound healing, remains limited. This calls for further investigation to elucidate its phytochemical constituents and *in vitro* antioxidant properties as preliminary steps in drug discovery before advanced preclinical studies.

Accordingly, this study was designed to qualitatively and quantitatively analyse the leaf extract of *Gossypium barbadense* by evaluating its *in vitro* antioxidant activity and validating its anti-ulcer potential through *in silico* analysis.

1.1. Hypothesis and Objective

We hypothesise that GBLE will have antioxidant and anti-ulcer potential against oxidative stress-related diseases. The study, therefore, aimed to qualify and quantify *Gossypium barbadense* L. Leaf Extract and also evaluate its antioxidant and anti-ulcer potential using *in vitro* and *in silico* Approaches.

2. MATERIALS AND METHODS

2.1. Materials

Absolute ethanol, 2,2'-Azinobis (3-Ethylbenzothiazoline-6-sulphonic (ABTS), Folin-Ciocalteu reagent, aluminium chloride, distilled water, iron (III) Chloride (FeCl_3), Griess reagent, methanol, potassium persulphate, DPPH (2,2'-diphenyl-1-picrylhydrazyl) reagent, sodium carbonate, hydrochloric acid, ascorbic acid, tannic acid, chloroform, sodium hydroxide, and sodium dodecyl sulphate were product of Sigma-Aldrich (St. Louis, USA), while all other laboratory reagent used were of analytical grade.

2.2. Plant Collection and Authentication

Gossypium barbadense leaves were sourced from Ikirun town, Osun State, Nigeria. A Botanist identified these leaves at the Department of Plant Biology, Osun State University, Osogbo, Nigeria, and a voucher specimen (Voucher No. 2026020001) was deposited in the herbarium.

2.3. Preparation of GBLE

At room temperature, the plant's fresh leaves were air-dried, pulverised using an electric blender to obtain a fine powder, and then stored in an airtight container to preserve their integrity until further use. 600 g of powdered *G. barbadense* leaves was macerated in 3.6 litres of absolute ethanol (1:6 w/v) for 14 days

to extract bioactive phytochemicals. The resulting mixture was filtered, and the filtrate was concentrated at 40°C using a rotary evaporator. The resulting residue was further dried in a water bath to obtain a concentrated crude extract, which was refrigerated for later use.

2.4. Qualitative and Quantitative Phytochemical Analysis of the GBLE

In accordance with the method of Odebiyi and Sofowora, (1978), the presence of the following phyto-bioactive compounds, flavonoids, saponins, glycosides, tannins, triterpenes and xanthoprotein were qualitatively assessed, while their quantitative analysis was conducted following their respective methods; Glycoside content was determined by a colorimetric reaction between the deoxysugar portion of cardiac glycosides and specific reagents in an acidic medium which produced a coloured complex that was spectrophotometrically measured at 495 nm following the method of Harborne (1998); the formation of blue-coloured phosphomolybdic-phosphotungstic acid complexes on reaction with Folin-Ciocalteu reagent was used for tannins determination, which was measured spectrophotometrically (Swain, 1979); Aluminium chloride colorimetric method was employed for determination of total flavonoid content, which resulted in formation of stable acid complexes on reaction with either the hydroxyl groups of carbon-3 (C-3) of flavonols or flavone and its carbon-4 (C-4) keto group as reported by Kaufman *et al.* (1999); Saponins content was quantified spectrophotometrically using the method of Brunner (1984) which involved the formation of blood red coloured products formed by the reaction of the oxidised triterpene of saponin with iron (III) Chloride; Triterpene was determined using Liebermann-Burchard reaction which involved of a reagent mixture containing acetic anhydride and concentrated sulfuric acid with triterpene to produce a coloured complex whose absorbance is proportional to the triterpene's concentration in GBLE (Harborne, 1998) and Xanthoprotein contents measurement is based on the nitration of aromatic amino acids present in proteins by concentrated nitric acid, forming yellow nitro derivatives whose intensity was measured spectrophotometrically (Wilson & Walker, 2000).

2.5. In Vitro Antioxidant Assay of the *G. barbadense* Leaf Extract (GBLE)

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was determined spectrophotometrically by measuring the absorbance of the yellow-coloured products formed upon reducing the DPPH radical with the antioxidants present in the GBLE at 517 nm, which aligned with the prior method of Brand-Williams. (1995). Briefly, varying concentrations of the extract (50-500 µg/ml) and the standard (ascorbic acid) were prepared in distilled water, mixed with a methanolic DPPH solution (0.5 ml, 0.1 M), shaken, and incubated in the dark at room temperature for 30 minutes. Upon spectrometric measurement, the results were expressed as percentages of DPPH scavenging Effect.

The 2,2'-Azinobis (3-Ethylbenzothiazoline-6-Sulphonic acid) (ABTS) scavenging activity of GBLE was determined by measuring the level at which compounds in the extract neutralise the ABTS⁺ radical, resulting in a spectrophotometric decrease in colour intensity at 734 nm (Re *et al.*, 1999). For this, varying concentrations of 0.01-5.0 mg/ml of GBLE and 0.01- 5.0 mg/ml of ascorbic acid (standard) were prepared and mixed with 2.0 ml of diluted ABTS radical cation solution. The result was expressed as the percentage of ABTS scavenging effect.

In accordance with the protocol of Bhaskar & Balakrishnan (2009), the Nitric Oxide (NO) scavenging activity was measured by oxidation of nitric oxide produced from sodium nitroprusside by GBLE.

Briefly, the GBLE solution at varying concentrations (1-20 µg/ml) in distilled water was mixed with 1 ml of 10 mM sodium nitroprusside and phosphate buffer (pH 7.4), incubated, and treated with 1 ml of Griess reagent. The mixture was kept standing for 5-10 minutes, and the absorbance of the coloured product formed was measured at 540 nm.

Hydrogen peroxide radical scavenging activity was determined spectrophotometrically at 230 nm by measuring GBLE's ability to donate electrons to Hydrogen peroxide, thereby decomposing it into water (H₂O) and Oxygen (O₂), thereby reducing the concentration in the reaction mixture (Gülçin *et al.*, 2002).

2.6. GC-MS analysis of GBLE

GBLE was characterised using a 7890B gas chromatograph coupled to a 5977C inert mass spectrometer with an electron impact source (Agilent Technologies). GBLE sample (2.5 g) was extracted by adding 20ml of ethanol and sonicating in an ultrasonic bath. It was cleaned up with a mixture of graphite, carbon black, Magnesium sulphate, and a primary-secondary amine to remove the matrix pigment. The mass spectrometer was operated at 70 eV in electron-impact ionisation mode with an ion source temperature set at 230°C, a quadrupole temperature of 150°C, and a transfer line temperature of 280°C. Possible compounds were scanned within a mass-to-charge ratio (m/z) range of 50 to 550 amu at a scan speed of 2.62 s/scan rate, and the identification of compounds was carried out by comparing the obtained mass spectral data with standard reference spectra available in the NIST 14 Mass Spectral Library.

2.7. In Silico Studies

The three-dimensional (3D) structures of the selected ligands identified from *Gossypium barbadense* leaf extract (GBLE) were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in Structure Data File (SDF) format. The ligand structures were converted to Protein Data Bank (PDB) format using Open Babel and subjected to energy minimisation to optimise their geometries and reduce structural strain. The minimised ligands were subsequently converted to PDBQT format using AutoDock Tools for molecular docking.

The three-dimensional structures of the selected target proteins were obtained from the Protein Data Bank (PDB). Protein preparation was performed using Discovery Studio Visualizer by removing co-crystallised ligands, water molecules, and other non-essential heteroatoms. Polar hydrogen atoms were added, and Gasteiger partial charges were assigned using AutoDock Tools. The binding sites of the target proteins were defined based on the positions of co-crystallised ligands and/or previously reported active-site residues. The docking grid boxes were centred on the corresponding binding sites and adjusted to adequately encompass the active-site regions.

Molecular docking simulations were performed using AutoDock Vina through the PyRx platform. Each ligand was individually docked into the predefined binding pocket of its respective target protein. Unless otherwise specified, the docking parameters were maintained at their default settings. The predicted binding affinities were expressed in kcal/mol, and the top-ranked ligand conformations based on docking scores were selected for subsequent interaction analysis. The docking results were interpreted as predicted ligand–protein binding poses and affinities rather than as experimental evidence of binding.

Post-docking analysis was performed using Discovery Studio Visualizer to examine the predicted protein–ligand complexes and characterise the principal non-covalent interactions. These included hydrogen bonds, hydrophobic interactions, π – π interactions, and other relevant contacts between the ligands and amino acid residues within the binding sites.

In silico pharmacokinetic and drug-likeness properties of the selected ligands were evaluated using the SwissADME web server. The assessment included Lipinski's rule of five, gastrointestinal absorption, bio-availability, lipophilicity, and aqueous solubility. These computational predictions were used to complement the molecular docking analysis and provide an initial assessment of the drug-likeness and ADME characteristics of the selected GBLE-derived compounds.

2.8. Statistical Analysis

Data were analysed using a one-way analysis of variance (ANOVA), followed by a post hoc Bonferroni multiple comparisons test to assess differences between mean values. The results were expressed as mean $\pm$ standard deviation (SD). All analyses were carried out using GraphPad Software Prism.

3. RESULTS

3.1. Extraction Yield of GBLE

The percentage yield of GBLE upon extraction was calculated using the formula:

$$Yield (\%) = \frac{\text{Mass of Extract Obtained}}{\text{Mass of plant material used}} \times 100 \quad \dots\dots (1)$$

Given: Initial plant material weight of 600 g,

Mass of extract obtained = 31.68 g,

Mass of plant material used = 600 g

$$\begin{aligned} Yield (\%) &= \frac{31.68}{600} \times 100 \\ &= 0.0528 \times 100 \\ &= 5.28\% \end{aligned}$$

The crude extract yield was about 5.28 % of the sample (600 g).

The phytoactive compounds identified and quantified in GBLE, along with their respective compositions, are highlighted in Table 1 below. The findings indicated the presence of bioactive compounds, including xanthoproteins, glycosides, tannins, saponins, and flavonoids, in the plant. Xanthoprotein has the highest value (73.90 ± 42.60 g/100g), followed by saponins (64.05 ± 36.92 g/100g), glycosides (41.57 ± 23.96 g/100g), flavonoids (4.94 ± 2.85 g/100g), and Triterpenes (3.45 ± 1.99 g/100g), while tannin has the lowest value (1.97 ± 1.14 g/100g).

Table 1. Quantitative phytochemical composition of GBLE.

Phytobioactive Compound	Crude Calculated (g/100 g)
Flavonoids	4.94 ± 2.85
Triterpenes	3.45 ± 1.99
Glycosides	41.57 ± 23.96
Tannin	1.97 ± 1.14
Saponin	64.05 ± 36.92
Xanthoprotein	73.90 ± 42.60

Figure 1 compared the DPPH radical scavenging inhibition of GBLE to a standard antioxidant compound (Ascorbic acid), n=3. At the chosen concentrations, GBLE scavenged DPPH radicals more effectively than ascorbic acid. At concentrations of 1, 2, 5, 10, and 20 µg/ml, the percentage scavenging inhibition of GBLE was 62.5, 75.6, 83.80, 85.60, and 91.30%, while that of the standard was 8.13, 68.13, 77.50, 81.25, and 85.00%, respectively. The DPPH's half concentration inhibition (IC_{50}) is 2.36, while that of Ascorbic acid is 31.50.

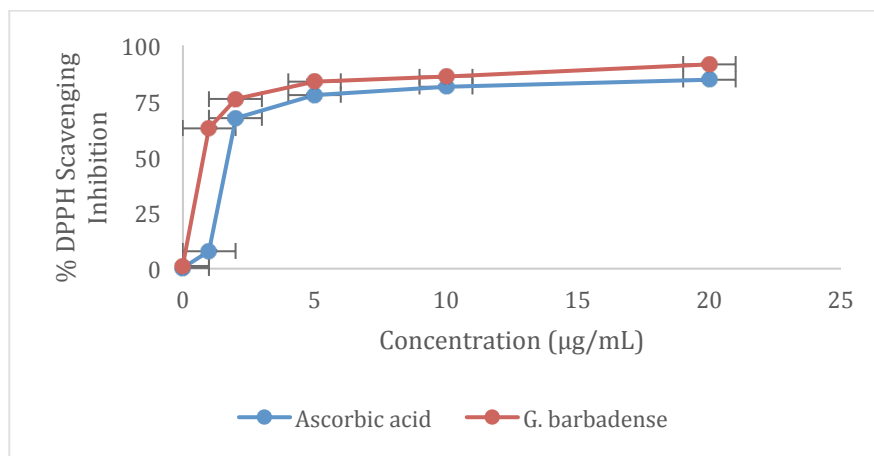


Figure 1: DPPH Radical Scavenging Inhibition of GBLE and Ascorbic acid

Figure 2 presents the nitric oxide radical scavenging inhibition of GBLE and ascorbic acid, $n = 3$, at varying concentrations ranging from 1–20 $\mu\text{g/mL}$. At all tested concentrations, GBLE exhibited greater nitric oxide radical scavenging activity, expressed as percentage inhibition, than ascorbic acid. At concentrations of 1, 2, 5, 10, and 20 $\mu\text{g/mL}$, the percentage inhibition of nitric oxide radicals by GBLE was 32.4, 49.00, 64.80, 74.50, and 80.70 %, respectively. In comparison, the corresponding inhibition values for ascorbic acid were 11.03%, 42.76%, 61.38%, 71.03%, and 78.62%, respectively. Furthermore, the IC_{50} value for GBLE was determined to be 15.55 $\mu\text{g/mL}$, whereas that of ascorbic acid was 62.16 $\mu\text{g/mL}$.

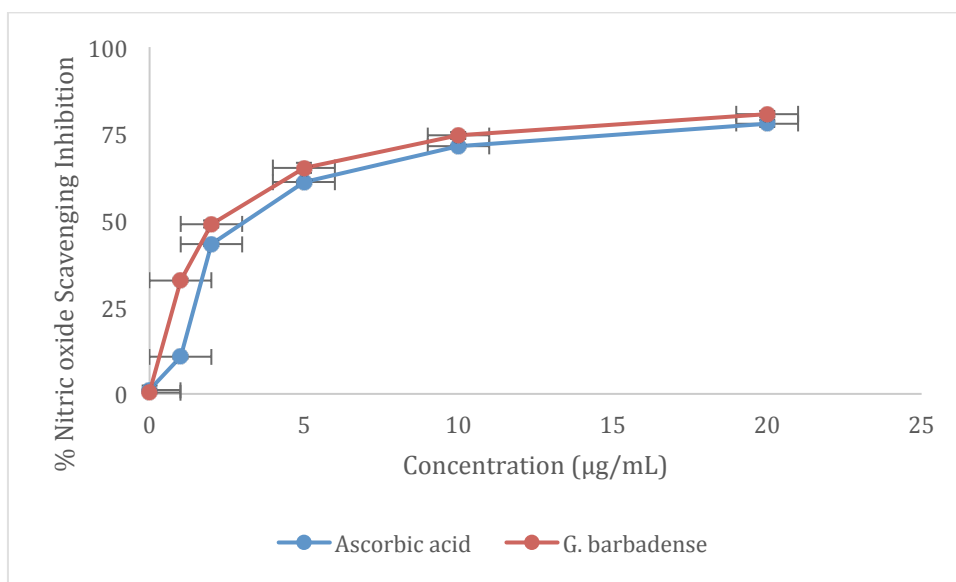


Figure 2: Nitric Oxide Radical Scavenging Inhibition of GBLE and Ascorbic Acid

Figure 3 illustrates the hydrogen peroxide scavenging inhibition of GBLE compared with ascorbic acid, $n = 3$, at varying concentrations. Similar to the nitric oxide radical-scavenging assay, the hydrogen peroxide-scavenging inhibition of GBLE, expressed as percentage inhibition, was higher than that of ascorbic acid across all tested concentrations. At concentrations of 1, 2, 5, 10, and 20 $\mu\text{g/mL}$, the percentage inhibition of hydrogen peroxide by GBLE was 14.1, 38.30, 74.00, 77.20, and 81.10%, respectively. In comparison, the corresponding inhibition values for ascorbic acid were 10.68%, 36.89%, 64.56%, 71.85%, and 77.18%, respectively. However, the estimated IC_{50} value for GBLE (60.63 $\mu\text{g/mL}$) was greater than that of ascorbic acid (28.2 $\mu\text{g/mL}$), indicating that ascorbic acid exhibited stronger scavenging potency despite the higher percentage inhibition observed for GBLE at the tested concentrations.

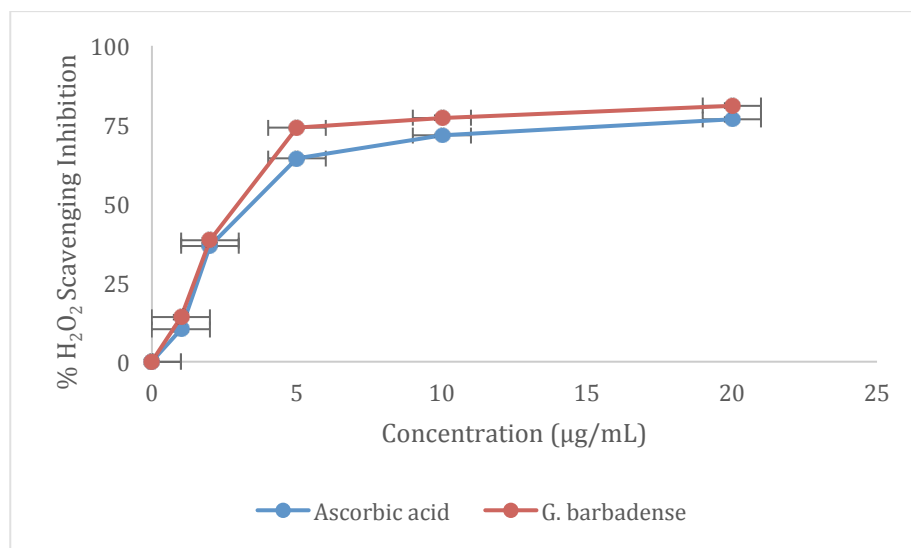


Figure 3: H_2O_2 Scavenging Inhibition of GBLE and Ascorbic acid

Figure 4 presents the 2, 2'-Azinobis (3-Ethylbenzothiazoline-6-Sulphonic acid (ABTS) radical scavenging inhibition of GBLE compared with ascorbic acid, $n=3$, at varying concentrations. The results show that GBLE exhibited markedly greater ABTS radical-scavenging inhibition than ascorbic acid across all concentrations tested. At concentrations of 1, 2, 5, 10, and 20 $\mu\text{g/mL}$, the percentage inhibition of the ABTS radical by GBLE was 56.30, 68.90, 78.80, 85.40, and 88.10 %, respectively. In contrast, the corresponding inhibition values for the standard antioxidant, ascorbic acid, were 8.61%, 37.09%, 59.70%, 67.55%, and 81.46%, respectively. However, the estimated IC_{50} value for GBLE was 3.43 $\mu\text{g/mL}$, while that of ascorbic acid was 83.05 $\mu\text{g/mL}$. Thus, GBLE showed stronger antioxidant activity than the standard.

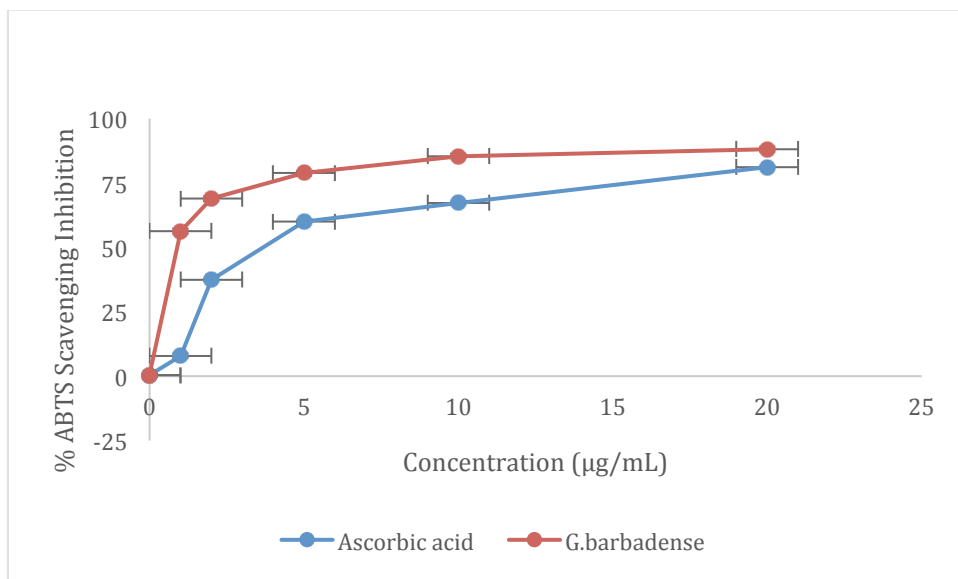


Figure 4: ABTS Radical Scavenging Inhibition of GBLE and Ascorbic acid

3.2. Determination of IC_{50}

The IC_{50} was defined as the concentration of the test sample required to inhibit 50% of the measured biological activity. IC_{50} values were expressed in the corresponding concentration units and used to compare the inhibitory activities of the tested samples. The half-maximal inhibitory concentration (IC_{50}) was determined using GraphPad Prism 9.0 (GraphPad Software, USA). Concentration-response data were plotted as percentage inhibition versus the corresponding test concentrations and fitted by nonlinear regression using a variable-slope four-parameter logistic model.

3.3. GC-MS Results of GBLE

Table 2 presents a list of selected therapeutic phytobioactive compounds identified in GBLE through qualitative characterisation and Gas Chromatography-Mass Spectrometry analysis, with each compound's molecular formula, molecular weight, area, retention time (RT), and chemical class provided.

Table 2. GC-MS of GBLE

Peak No	Compound	Area %	Retention Time (RT), min	Molecular weight (amu)	Molecular Formula	Chemical Class
1	Eicosane, 10-methyl	1.20	4.605	296.344	$\text{C}_{21}\text{H}_{44}$	Branched Alkane
2	Tetracosane, 3-Ethyl	2.53	4.773	366.423	$\text{C}_{26}\text{H}_{54}$	
3	Heptadecane, 9-octyl	0.91	6.396	352.407	$\text{C}_{25}\text{H}_{52}$	
4	Heneicosane	1.19	6.490	296.344	$\text{C}_{21}\text{H}_{44}$	
5	Alpha-copaene	2.67	5.569	204.188	$\text{C}_{15}\text{H}_{24}$	Sesquiterpene

Peak No	Compound	Area %	Retention Time (RT), min	Molecular weight (amu)	Molecular Formula	Chemical Class
6	Caryophyllene	14.85	5.946	204.188	C ₁₅ H ₂₄	
7	Humulene	4.43	6.239	204.188	C ₁₅ H ₂₄	
8	Dodecanoic acid, methyl ester	3.30	6.877	214.193	C ₁₂ H ₂₄ O ₂	Fatty acid
9	Methyl tetradecanoate	2.90	8.594	242.2256	C ₁₅ H ₃₀ O ₂	
10	Hexadecanoic acid, methyl ester	8.51	10.154	270.256	C ₁₇ H ₃₄ O ₂	
11	9-Octadecenoic acid, methyl ester (E)-	5.20	11.358	296.272	C ₁₉ H ₃₆ O ₂	
12	Methyl stearate	1.29	11.568	298.287	C ₁₉ H ₃₈ O ₂	

GC-MS analysis revealed 12 compounds from GBLE that are of therapeutic benefit, as in Table 2. These compounds are Methyl tetradecanoate (2.90%), Eicosane, 10-methyl (1.20%), Tetracosane, 3-Ethyl (2.53%), Alpha-copaene (2.67%), Hexadecanoic acid, methyl ester (8.51%), β-Caryophyllene (14.85%), Heptadecane, 9-octyl (0.91%), Heneicosane (1.19%), Dodecanoic acid, methyl ester (3.30%), Methyl stearate (1.29%), 9-Octadecenoic acid-methyl ester (E) (5.20%), humulene (4.43%).

Figure 5 presents the GC-MS Chromatogram obtained from the analysis, showing the various peaks with their retention times and abundances. However, among these bioactive compounds, two compounds with the prominent peaks, named β-Caryophyllene (14.85%) and Hexadecanoic acid- methyl ester (8.51%) have been previously documented to exhibit antibacterial properties (Constani *et al.*, 2019), antioxidant and analgesic properties (Machado *et al.*, 2020), gastroprotective activity (Sharma *et al.*, 2023) and anti-inflammatory properties (Francomano *et al.*, 2019; Rodríguez-Mejía *et al.*, 2022; Dinku *et al.*, 2022),

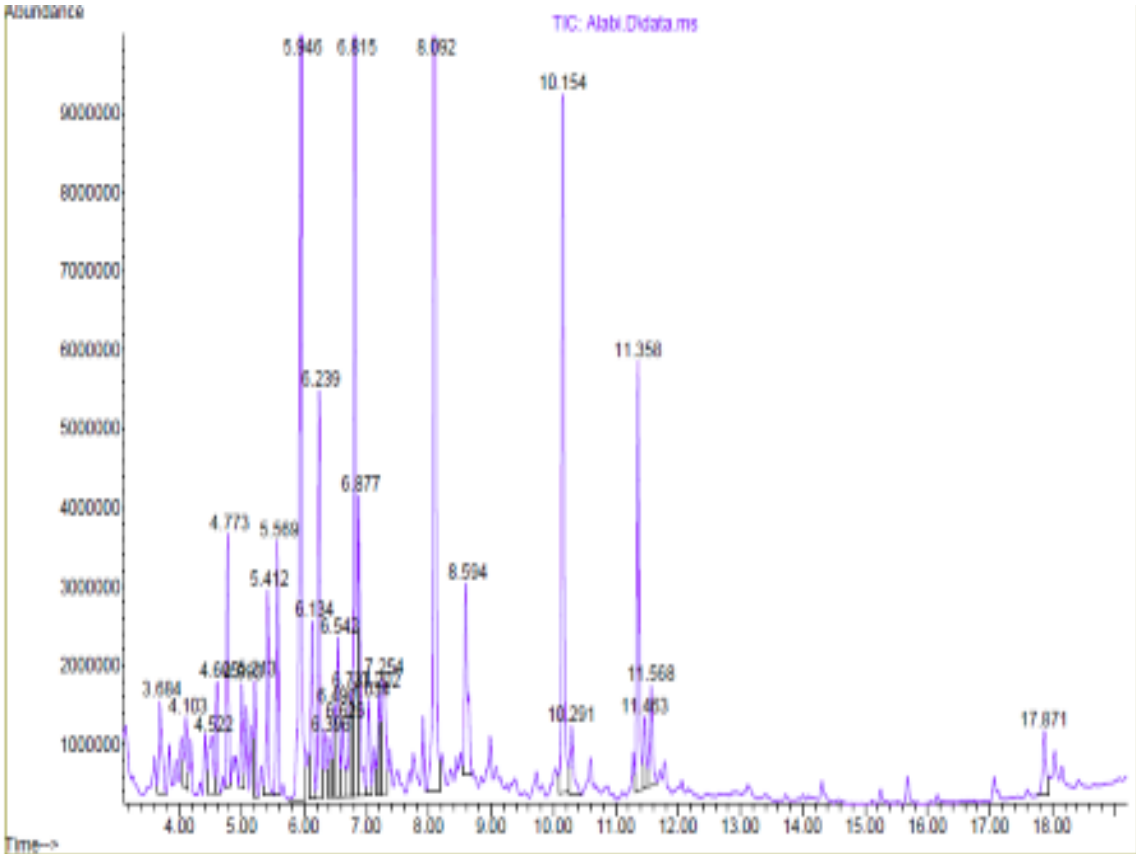


Figure 5: GC-MS Chromatogram and Identified Bioactive Compounds in GBLE

3.4. *In Silico* Results and Molecular Docking

Table 3. GBLE ligands with 1NFK.

Ligand	Binding Affinity (kcal/mol)
1VX	-7.5
Caryophyllene	-5.7
Alpha-copaene	-5.7
Pepstatin	-5.5
Humulene	-5.4
Octadecane, 5-methyl-	-4.6
9-Octadecenoic acid, methyl ester, (E)-	-4.2
Dodecanoic acid, methyl ester	-4.0
Eicosane, 10-methyl-	-3.9
Hexadecanoic acid, methyl ester	-3.9
Heptadecane, 9-octyl-	-3.8
Heneicosane	-3.5
Methyl stearate	-3.5
Tetracosane, 3-ethyl-	-3.4

Table 4. GBLE with IPSO.

Ligand	Binding affinity (kcal/mol)
Pepstatin	-8.4
1VX	-7.3
Alpha-copaene	-5.9
Heptadecane, 9-octyl-	-5.6
Methyl stearate	-5.6
Methyl tetradecanoate	-5.5
Hexadecanoic acid, methyl ester	-5.4
Eicosane, 10-methyl-	-5.4
Humulene	-5.2
Caryophyllene	-5.1
Tetracosane, 3-ethyl-	-5.1
Dodecanoic acid, methyl ester	-5.0
Heneicosane	-4.2
9-Octadecenoic acid, methyl ester, (E)-	-4.0

Table 5. GBLE with 4L7D.

Ligand	Binding affinity (kcal/mol)
1VX	-10.6
Pepstatin	-8.2
Caryophyllene	-7.0

Ligand	Binding affinity (kcal/mol)
Humulene	-6.9
Alpha-copaene	-6.6
Heptadecane, 9-octyl-	-5.4
9-Octadecenoic acid, methyl ester (E)-	-5.2
Heneicosane	-5.2
Tetracosane, 3-ethyl-	-5.1
Methyl stearate	-5.0
Hexadecanoic acid, methyl ester	-4.9
Eicosane, 10-methyl-	-4.9
Methyl tetradecanoate	-4.9
Dodecanoic acid, methyl ester	-4.8

Table 6. Physicochemical properties of GBLE ligands with Pepstasin and IVX

Parameters	Compounds							
	Alpha-copaene	Caryophyllene	Humulene	9-Octadecenoic acid, methyl ester (E)-	Methyl stearate	Tetracosane, 3-ethyl-	Pepstasin	IVX
Molecular Formula	C ₁₅ H ₂₄	C ₁₅ H ₂₄	C ₁₅ H ₂₄	C ₁₉ H ₃₆ O ₂	C ₁₉ H ₃₈ O ₂	C ₂₆ H ₅₄	C ₃₄ H ₆₃ N ₅ O ₉	C ₂₇ H ₃₀ N ₂ O ₄
Molecular weight (g/mol)	204.35	204.35	204.35	296.49	298.50	366.74	685.54	446.54
Number of heavy atoms	15	15	15	21	21	26	48	33
Number of aromatic Atoms	0	0	0	0		0	0	12
Topological polar surface area (TPSA)	0	0	0	26.3 Å	26.3 Å	0	223.26 Å	77.92 Å
Fraction Csp3	0.87	0.73	0.60	0.84	0.95	1	0.82	0.44
Number of rotatable bonds	1	0	0	16	17	22	27	5
Number of H-bond acceptors	0	0	0	2	2	0	9	4
Number of H-bond donors	0	0	0	0	0	0	8	1
Molecular refractivity	67.14	68.78	70.42	94.26	94.73	127.1	184.66	133.34

Table 7. Lipophilicity and water solubility of GBLE ligands with Pepstasin and IVX

Compounds	Lipophilicity	Water Solubility					
	Consensus log P _{o/w}	Log S (ESOL)	Solubility Class	Log S (all)	Solubility Class	Log S (sili- cos-it)	Solubility Class
Alpha-copaene	3.62	-3.86	Soluble	-4.19	Moderately soluble	-3.07	Soluble
Caryophyllene	4.24	-3.87	Soluble	-4.1	Moderately soluble	-3.77	Soluble
Humulene	4.26	-3.97	Soluble	-4.27	Moderately Soluble	-3.52	Soluble
9-Octadecenoic acid, methyl ester (E)-	5.95	-5.32	Moderately solu- ble	-7.83	poorly Soluble	-6.09	Poorly solu- ble
Methyl stearate	6.24	-5.83	Moderately solu- ble	-8.77	Poorly soluble	-6.81	Poorly solu- ble
Tetracosane, 3-ethyl-	10.05	-9.49	Poorly soluble	-14.09	Insoluble	-9.49	Poorly solu- ble
Pepstatin	2.37	-4.19	Moderately solu- ble	-7.34	Poorly soluble	-4.55	Moderately soluble
IVX	3.26	-4.72	Moderately solu- ble	-4.77	Moderately soluble	-5.83	Moderately soluble

Table 8. Pharmacokinetics of GBLE ligands with Pepstasin and IVX

PARAMETERS	COMPOUNDS							
	α - copaene	Caryophyllene	Humulene	9- Octadecenoic acid, methyl ester (E)-	Methyl stearate	Tetracosane, 3-ethyl-	Pepstasin	IVX
Blood-brain barrier permeation	Yes	No	No	No	No	No	No	Yes
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.11	0.56
Gastrointestinal ab- sorption	Low	Low	Low	High	High	Low	Low	High
P-glycoprotein sub- strate	No	No	No	No	No	Yes	No	Yes
Cytochrome P450 1A2 inhibitor	No	No	No	Yes	Yes	No	No	No
Cytochrome P450 2C19 inhibitor	Yes	Yes	No	No	No	No	No	No
Cytochrome P450 2C9 inhibitor	No	Yes	Yes	No	No	No	No	Yes
Cytochrome P450 3A4 inhibitor	No	No	No	No	No	No	No	Yes
Cytochrome P450 2D6 inhibitor	No	No	No	No	No	No	No	Yes

Table 9. Drug-likeness and medicinal chemistry

COMPOUNDS	Drug-likeness rules					Medicinal Chemistry			
	Lipinki #violations	Ghose #violations	Veber #violations	Egan #violations	Muegge #violations	PAINS #alerts	Brenk #alerts	Lead likeness #violations	Synthetic accessibility
Alpha-copaene	1	0	0	0	1	0	1	2	4.62
Caryophyllene	1	0	0	0	1	0	1	2	4.51
Humulene	1	0	0	0	1	0	1	2	3.66
9-Octadecenoic acid, methyl ester (E)-	1	1	1	1	2	0	1	2	3.16
Methyl stearate	1	1	1	1	2	0	0	2	2.76
Tetracosane, 3-ethyl-	1	2	1	1	3	0	0	3	3.44
Pepstatin	3	3	2	1	4	0	0	2	6.65
IVX	0	1	0	0	0	0	0	1	4.11

Table 10. Summary of the docking scores of the six shortlisted ligands with Pepstatin and IVX across the three targets

Ligand	IPSO (kcal/mol)	INFK(kcal/mol)	4LFD(kcal/mol)	Observation
Pepstatin (standard)	-8.4	-5.5	-8.2	Best on IPSO, strong on 1NFK, and very strong on 4LFD
IVX (standard)	-7.3	-7.5	-10.6	Best on 1NFK, Best on 4LFD, and very strong on IPSO
Alpha-copaene	-5.9	-5.7	-6.6	strong on 1NFK, strong on IPSO, and good on 4LFD
Methyl stearate	-5.6	-3.5	-5.0	Weak across all proteins
Humulene	-5.2	-5.4	-6.9	Weak across all proteins
Caryophyllene	-5.1	-5.7	-7.0	Weak on IPSO, strong on 1NFK and 4LFD
Tetracosane, 3-ethyl-	-5.1	-3.4	-5.1	Weak across all proteins
9-Octadecenoic acid, methyl ester (E)-	-4.0	-4.2	-5.2	Weak across all proteins

4. DISCUSSION

The phytochemical analysis conducted on *G. barbadense* leaf extract, as represented in Table 1, revealed the presence of flavonoids, triterpenes, xanthoprotein, saponins, tannins and glycosides, which have been reported to exhibit antioxidant, anti-inflammatory, anti-viral properties, antitumor, anti-ulcer, gastroprotective, anti-convulsant, anti-diabetic, antibacterial, anthelmintic and anti-fertility activities (Vishwakarma *et al.*, 2022; Morales-Aranibar *et al.*, 2023; Alzandi *et al.*, 2025; Adib *et al.*, 2024).

Glycosides are compounds consisting of a glycosyl group and an aglycone (Johnson *et al.*, 2021), characterised by a sugar moiety linked to a non-sugar aglycone. They can exhibit diverse biological functions that are exploited for therapeutic benefit. Flavonoids are secondary metabolites which comprise a

benzopyrone ring bearing phenolic or polyphenolic groups at different positions (Cavalcante *et al.*, 2022). Both function as protective and regulatory secondary metabolites that help plants defend against pathogenic organisms and herbivores. Previous studies have shown that these compounds have been extensively utilised as anti-inflammatory, antimicrobial, antioxidant, antiproliferative, antitumor, antimalarial, antian-giogenic, anti-viral, anticancer, and neuroprotective agents (Zhao *et al.*, 2019; Atrahimovich *et al.*, 2021; Xie *et al.*, 2022) Figures 6-8.

Table 11. Summary of the key parameters used for the final selection of the ligands

Parameters	Alpha-copaene	Caryophyllene	Humulene	9-Octadecenoic acid, methyl ester (E)-	Methyl stearate	Tetracosane, 3-ethyl-	Pepstatin	IVX
Molecular weight (g/mol)	204.35	204.35	204.35	296.49	298.50	366.74	685.54	446.54
Topological polar surface area (TPSA)	0	0	0	26.3Å	26.3 Å	0	223.26 Å	77.92 Å
Gastrointestinal absorption	Low	Low	Low	High	High	Low	Low	High
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.11	0.56
Muegge Viola-tions	1	1	1	2	2	3	4	0
Lipinski viola-tions	1	1	1	1	1	1	3	0
Drug likeness	Good	Good	Good	Moderate	Moderate	Poor	Poor	Very Good

3.5. Results of the Receptor-ligand Interactions Between the Docked Ligands and Proteins

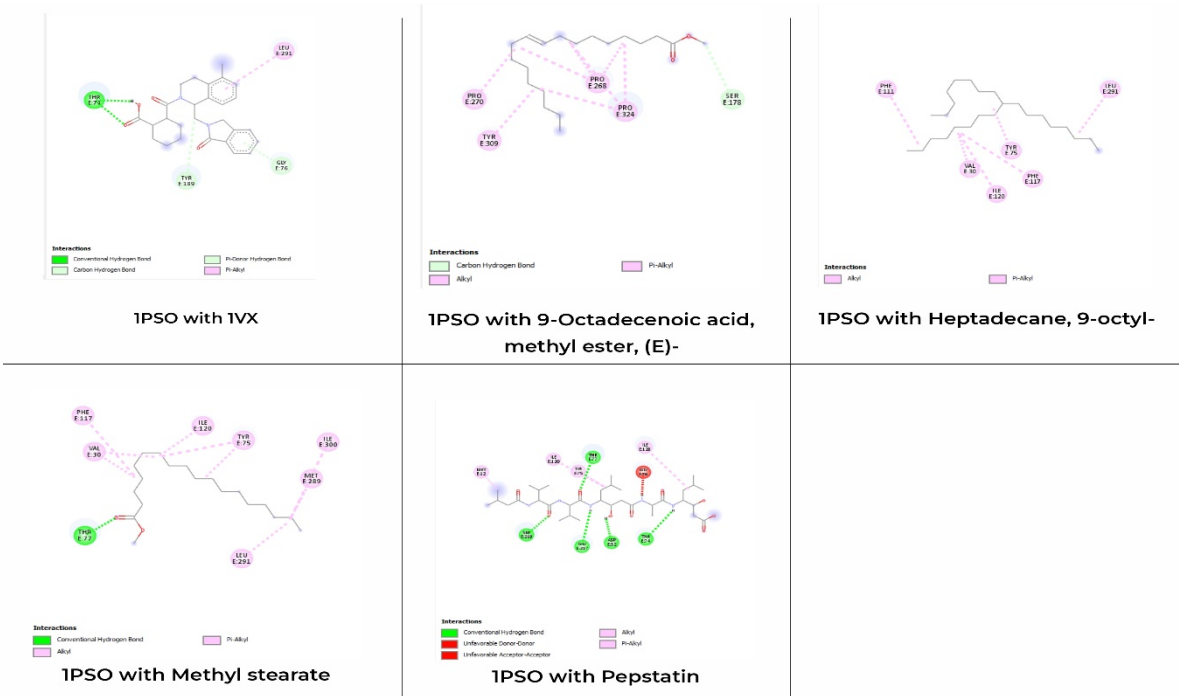


Figure 6: IPSO with GBLE Ligands and IVX and Pepstatin

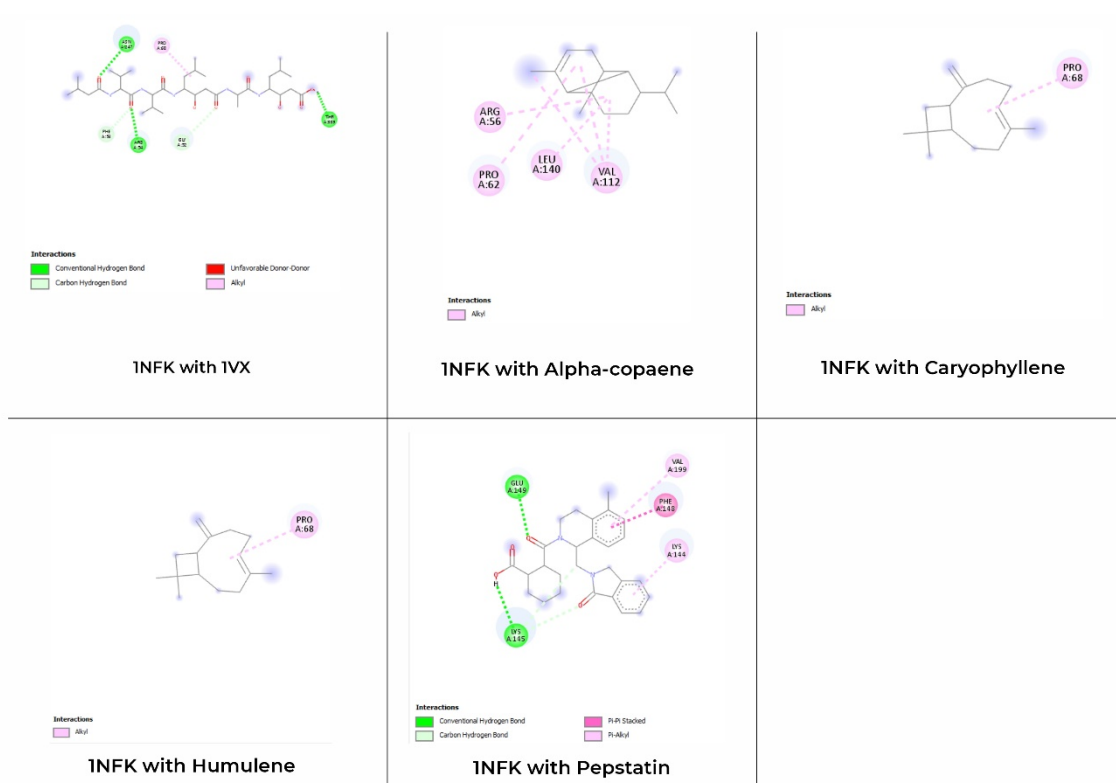
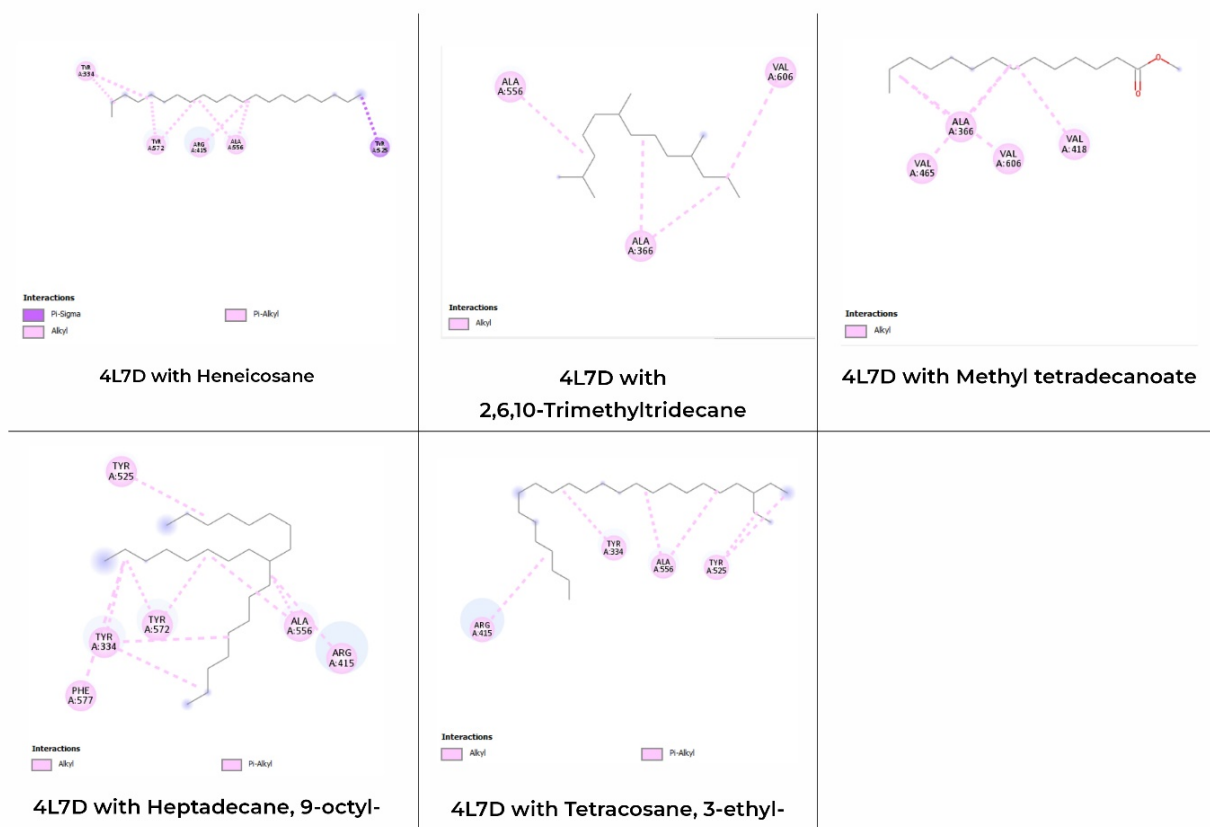
**Figure 7:** 1NFK with GBLE Ligands and IVX and Pepstatin**Figure 8:** 4LFD with GBLE Ligands and IVX and Pepstatin

Table 12. Choice of Target Proteins

SN	Protein Targets	Pathway	Mechanistic Role in Gastrotoxicity	Top Ligands
1.	1NFK	Gastric inflammation	Reduces immune –mediated gastric mucosal injury and pain	Alpha-copaene and Caryophyllene strong on 1NFK, strong on 1PSO, and good on 4LFD
2.	IPSO	Gastric proteolytic injury	Reduces direct chemical damage	
3.	4L7D	Oxidative stress	Increases antioxidant defense and healing	

The attachment of multiple phenolic hydroxyl groups to a conjugated aromatic ring system in flavonoids enables hydrogen atom or electron donation to neutralise reactive oxygen species and free radicals, thereby conferring strong antioxidant activity and contributing to other pharmacological effects, such as cardioprotective, antimicrobial, anti-ulcer, and anti-inflammatory properties. In addition, flavonoids can chelate transition metals such as iron and copper, thereby reducing oxidative stress and enhancing their therapeutic significance in medicinal plants (Ferraz *et al.*, 2020). However, the aglycone and glycone moieties of glycosides are primarily responsible for their therapeutic activity. While the sugar component improves solubility, stability, and bioavailability, the aglycone typically mediates the primary pharmacological Effect. Glycosides undergo enzymatic hydrolysis *in vivo*, releasing the active aglycone, which exerts diverse therapeutic effects depending on its chemical structure (Tang *et al.*, 2025; Xie *et al.*, 2022). Numerous plant-derived flavonoids and glycosides exhibit anti-ulcer and gastroprotective properties, including quercetin and rutin from *Allium cepa* and *Fagopyrum esculentum*, as well as geniposide from *Gardenia jasminoides* and salidroside from *Rhodiola rosea* (Liu *et al.*, 2022; Feng *et al.*, 2022).

Saponins are plant-derived glycosides with triterpenoid or steroidal aglycones, whereas tannins are high-molecular-weight polyphenols with strong protein- and metal-binding capacities. Both are secondary metabolites involved in plant defence and exhibit diverse pharmacological activities, including hypolipidaemic, antioxidant, hypoglycaemic, antimicrobial, antihypertensive, antiasthmatic, cardioprotective, and anti-inflammatory effects (Shi *et al.*, 2022; Sharma *et al.*, 2023; Cosme *et al.*, 2025). These bioactivities are largely attributed to their structural properties and redox-modulating capabilities. Tannins' pharmacological activity is primarily linked to their polyphenolic structure, which enables free-radical scavenging, protection against oxidative damage, and protein precipitation, thereby forming a protective layer on the gastric mucosa (Cosme *et al.*, 2025). The amphiphilic structure of saponins, consisting of a lipophilic aglycone and hydrophilic sugar chains (Juang *et al.*, 2020), enables anti-inflammatory, membrane-stabilising and cytoprotective effects. These findings support the extract as a plausible therapeutic candidate for managing ulcers and gastrointestinal disorders.

Triterpenes, which form the aglycone backbone of many saponins, may further enhance the anti-ulcer activity of this extract by modulating inflammatory pathways and reinforcing mucosal defence systems (Baltina *et al.*, 2022; Shen *et al.*, 2025). Triterpenes are C₃₀ isoprenoid compounds produced via the mevalonate pathway and exhibit pharmacological activity due to their pentacyclic structures and functional groups such as carboxyl and hydroxyl moieties. These compounds inhibit the synthesis of inflammatory mediators and reactive oxygen species by modulating key cellular signalling pathways, including NF-κB and oxidative stress pathways (Khan *et al.*, 2023; Darshani *et al.*, 2022).

Evidence suggests that triterpenoid saponins significantly inhibit gastric lesions and improve mucosal integrity in experimental models (Baltina *et al.*, 2022). The presence of xanthoproteins (aromatic amino acid-containing compounds) in GBLE may also contribute to its overall protection, antioxidant capacity, and support for tissue repair. Xanthoproteins are derived from aromatic amino acids such as tyrosine, tryptophan, and phenylalanine, whose aromatic rings undergo nitration and donate electrons to neutralise reactive oxygen species, thereby conferring cytoprotective and antioxidant properties.

Earlier reports have identified triterpenes such as oleanolic acid from *Olea europaea*, lupeol from *Mangifera indica*, betulinic acid from *Betula pendula*, and astragaloside IV from *Astragalus membranaceus* (Darshani *et al.*, 2022; Khan *et al.*, 2023; Li *et al.*, 2025), as well as xanthoproteins such as pro-

tein-derived peptides from *Glycine max* and aromatic amino acid-rich proteins from *Oryza sativa* (Chen *et al.*, 2019), which exhibit anti-ulcer and gastroprotective effects by suppressing inflammatory mediators and oxidative stress associated with gastric injury, while enhancing prostaglandin synthesis and gastric mucus secretion.

Collectively, flavonoids and glycosides in medicinal plants may synergistically contribute to gastro-protective effects through antioxidant and anti-inflammatory activities, enhanced mucus production, and the formation of a protective mucosal barrier. This multimodal action is consistent with the established mechanisms of action of plant-derived anti-ulcer agents. It supports GBLE's potential as a promising agent for managing gastric ulcers and other gastrointestinal disorders.

To further assess the antioxidant activity of GBLE and guide dose selection and optimisation *in vivo* studies, *in vitro* antioxidant assays were conducted using hydrogen peroxide (H_2O_2), DPPH, Nitric oxide (NO) radical scavenging and ABTS⁺ inhibition methods. The results of DPPH scavenging activity of GBLE versus the Ascorbic acid (Figure 1) and ABTS radical scavenging activity versus the Ascorbic acid (Figure 4) demonstrate that GBLE exhibits greater potency in scavenging DPPH and ABTS radicals than ascorbic acid, as the percentage radical scavenging at the selected concentrations was significantly higher than that of the ascorbic acid. This may be due to the electron- or hydrogen-donating capacity of bioactive constituents present in GBLE, such as tannins, flavonoids, triterpenes and saponins as identified in the qualitative and quantitative phytochemical screening (Table 1). This redox potential enables the GBLE to neutralise free radicals by stabilising their unpaired electrons. In addition, the half-maximal inhibitory concentration (IC_{50}) values further confirm the superior free radical-scavenging capacity of GBLE compared to ascorbic acid, with IC_{50} values of 3.43 (ABTS) and 2.36 (DPPH), relative to 83.05 and 31.50 for the reference standards, respectively. These findings indicate that GBLE exhibits higher efficacy and stronger antioxidant activity, requiring lower concentrations to achieve comparable effects, thereby suggesting its potential as a promising candidate for further drug discovery and development.

Consistent with the ABTS and DPPH results, the nitric oxide (NO) radical-scavenging and hydrogen peroxide (H_2O_2) inhibition activities of GBLE (Figures 2 and 3) were greater than those of the reference standard, ascorbic acid, at all tested concentrations. This indicates that GBLE is more potent at scavenging NO radicals, which are key mediators of inflammation and oxidative stress (Babatunde *et al.*, 2025), and at mitigating the cytotoxic effects of hydrogen peroxide than ascorbic acid. These findings suggest that GBLE possesses enhanced antioxidant and anti-inflammatory properties relative to ascorbic acid, which may be effective in attenuating peroxide-induced and nitrosative cellular damage. Overall, the pharmacological efficacy of GBLE demonstrated in these *in vitro* assays suggests its potential to promote wound healing, suppress oxidative stress and reduce inflammation in the gastrointestinal tract and other organs.

The results obtained agreed with the report by Hussen & Endalew (2023) on the *in vitro* antioxidant activity of GBLE, showing that it exhibits Nitric oxide, DPPH, ABTS radical-scavenging, and H_2O_2 -inhibitory activities.

Mounting evidence from *in vivo* studies has also shown that β -Caryophyllene inhibits the production of interleukin-17 (IL-17), nitric oxide, TNF- α , hydrogen peroxide, and interferon gamma (IFN- γ), thereby reducing the extent of macrophage infiltration in gastric injury (Francomano *et al.*, 2019). In addition, the lipid-lowering, anti-inflammatory, antioxidant, and antibacterial potential of Hexadecanoic acid methyl ester has been documented in another *in vivo* study (Elwekeel *et al.*, 2023). This evidence affirmed the therapeutic use of the plant extract for the management of malabsorption, gastrointestinal, and stomach disorders. Based on the phytoactive compounds identified by GC-MS analysis, this study further affirmed the antioxidant, free radical-scavenging activity, and potential of GBLE to suppress inflammatory responses. The finding also suggests that the extract may be further exploited for anti-ulcer activity via an *in vivo* study.

Table 12 shows the rationale for selecting the target proteins NF- κ B (p65 subunit) (INFK), PEPSIN A (IPSO), and KEAP-1 (4LFD) as the most relevant and mechanistically informative targets. The abbreviations (INFK, IPSO and 4LFD) represent the PDB IDs of the proteins.

NF- κ B was selected as a primary docking target for gastric ulcer because it is a central regulatory node in gastric inflammation (Saha *et al.*, 2022). Pepsin A was selected due to its direct involvement in gastric mucosal damage (Heda *et al.*, 2023), while Keap1 was selected as the representative oxidative stress-related target due to its regulatory role in the Nrf2 antioxidant signalling pathway (Verza *et al.*, 2025). In summary, INFK, IPSO and 4LFD were selected as molecular docking targets because they directly correspond to the key pathological processes modulated *in vivo*, namely inflammation, gastric proteolytic injury, and oxidative stress. This strategic selection ensures a focused, mechanistically consistent, and molecular-docking analysis that complements the experimental findings.

By combining docking results with physicochemical parameters, lipophilicity, water solubility, pharmacokinetics, and druglikeness/medicinal chemistry traits as seen in Tables 6-11, ligand selection was improved. Molecular docking of the 12 initially selected GBLE ligands against the three target proteins produced binding affinities that range from -7.0 to -3.4 kcal/mol, while Pepstatin and IVX standards exhibited comparatively stronger affinities (-10.6 to -5.5 kcal/mol), out of which 6 ligands with the best molecular docking scores were selected for further studies. The six ligands were selected for their high docking scores, which indicate predicted binding affinities for the target proteins. Through several interaction types, including Pi-Sigma bonds, conventional hydrogen bonds, and Pi-Alkyl bonds, the compounds interact with specific regions of the protein. Ligand selection was improved by combining docking results with physicochemical properties, pharmacokinetics, lipophilicity, water solubility, and drug-likeness/medicinal chemistry characteristics.

Variables such as molecular weight, topological polar surface area (TPSA), and lipophilicity (LogP) are predictive of oral absorption and membrane permeability; this multifactorial approach is well established in virtual screening pipelines (Çevik *et al.*, 2025).

Predictions of water solubility were deemed significant because even high-affinity ligands can have poor solubility, which can limit systemic exposure. In addition, predicted gastrointestinal absorption, bioavailability scores, and P-glycoprotein substrate status are examples of pharmacokinetic metrics that provide insight into the potential for systemic distribution (Murakami *et al.*, 2021).

Predictions regarding water solubility were considered significant, as poor solubility can limit systemic exposure even for high-affinity ligands (Basharat *et al.*, 2025). Pharmacokinetic parameters, including predicted gastrointestinal absorption, bioavailability scores, and P-glycoprotein substrate status, provide insight into the likelihood of effective systemic delivery (Nguyen *et al.*, 2021). *In silico* CYP450 inhibition profiling was employed to predict metabolic stability and potential drug-drug interactions (Dmitriev *et al.*, 2021). The cytochrome P450 enzyme system is crucial in drug metabolism and elimination in biological systems.

Compounds with undesired structural liabilities or low development potential were eliminated using medicinal chemistry filters (PAINS, Brenk alerts) and drug-likeness rules (Muegge, Lipinski, Ghose, Egan, Veber) (Mahmud *et al.*, 2022). Hence, from this combined evaluation, Caryophyllene and α -copaene emerged as the top candidates, each exhibiting favourable ADMET properties and consistently high binding affinities across all targets. Previous studies have reported some biological activities of both compounds, including antioxidant and analgesic properties (Machado *et al.*, 2020), gastroprotective activity (Sharma *et al.*, 2023), and anti-inflammatory properties (Francomano *et al.*, 2019; Rodríguez-Mejía *et al.*, 2022; Dinku *et al.*, 2022). The present study revealed that neither compound inhibits the majority of Cytochrome P450 enzymes, indicating significant potential for bioavailability and transformation upon oral administration without causing adverse responses, as supported by (Saravanakumar *et al.*, 2019).

From the *in silico* results, Caryophyllene and α -copaene are likely to exhibit better gastro-protective effects than the conventional standards pepstatin and IVX (standards) in gastric ulcer healing due to their reduced toxicity, strong anti-inflammatory properties, improved mucosal regeneration and protection, increased safety, multimodal activity and antioxidant properties (Bolat *et al.*, 2025). This positions them as promising candidates for anti-ulcer therapeutics.

CONCLUSION

The present study evaluated the antioxidant activity and possible gastroprotective potential of *Gossypium barbadense* leaf extract using an *in silico* approach. *In vitro* antioxidant assays demonstrated appreciable free radical-scavenging activity, indicating the potential of the extract to mitigate oxidative stress. GC–MS profiling identified several bioactive compounds, while molecular docking predicted favourable interactions between selected compounds and target proteins, suggesting their possible contribution to the observed bioactivities of the extract.

Docking Validation

The docking protocol was evaluated retrospectively using the pepsin–pepstatin complex (PDB ID: 1PSO) as the reference structure. Redocking of the crystallographic pepstatin using PyRx/AutoDock Vina yielded a binding affinity of -8.4 kcal/mol, consistent with the initial docking result. Comparable redocking validation could not be performed for the NF- κ B and Keap-1 targets because suitable co-crystallised reference ligands were not available in the structures used. Accordingly, the docking results for these targets are considered predictive and should be interpreted with appropriate caution.

AUTHORS' CONTRIBUTIONS

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None

CONFLICT OF INTEREST

The author confirms that the content of this article has no conflicts of interest.

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