

Antioxidant and Hepatoprotective Activity of Phytoconstituents Isolated from *Euphorbia hirta* Leaves: An Integrated *in Vitro* and *in Silico* Approach

Johnson Gritto Michael Soundaraj^{1*}, Nithyatharani Ramalingam^{2#}, Manikandan Ramasamy³,
Sujeet Kumar Mritunjay⁴ and Cecileya Jasmin⁵

^{1*}Department of Botany, St. Xavier's College (Autonomous), Palayamakottai,
Tamil Nadu, India

²Department of Microbiology, Shrimati Indira Gandhi College, Tiruchirappalli,
TamilNadu, India

³Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli,
TamilNadu, India

⁴School of Sciences, Department of Microbiology, PP Savani University,
Kosamba, Surat, Gujarat, India

⁵Department of Biochemistry, Saveetha Medical College and Hospital, Saveetha Institute of Medical
and Technical Sciences (Deemed to be University), Thandalam, Chennai, Tamil Nadu, India and
Department of Biochemistry, Chettinad Institute of Medical Education and Research, Chettinad
Academy of Research and Education, BEML Nagar, Kolar Gold Fields, Karnataka, India

KEYWORDS Antioxidant Evaluation. *Euphorbia Hirta*. Hepatitis B Virus X Protein. Hepatoprotective Potential. Molecular Docking

ABSTRACT The hepatoprotective potential of *Euphorbia hirta* ethanol leaf extract was studied in this research. The leaves that were collected were washed followed by drying then powdered and extracted with ethanol. The findings revealed that phenolics, flavonoids, and tannins, which have significant antioxidant activity, may inhibit oxidative stress and free radical damage in liver cells. Gas chromatography study revealed various bioactive compounds in the extract. Molecular docking studies showed that some ligands had good binding affinity for the Hepatitis B virus X protein, which is associated with liver disease. Based on the findings, *Euphorbia hirta* could be a hepatoprotective and therapeutic option. Based on the study results, the plant can be viewed as a potential natural source for the development of liver-protective agents after further studies through *in vitro*, *in vivo* and clinical studies.

INTRODUCTION

Chronic viral hepatitis, particularly Hepatitis B virus (HBV) infection, remains a major cause of liver-related illness and death in various communities, making liver toxicity a significant global health issue (World Health Organisation 2023). The Hepatitis B virus X protein (HBx) plays a crucial role in HBV's ability to cause disease. It regulates viral replication, alters immune signalling in the host, and begins liver cell damage, leading to conditions like cirrhosis and liver cancer (Bouchard and Schneider 2004; Rawat and Bouchard 2015). Problems like drug resistance and side effects from current treatments, such as nucleoside analogues and type-I interferons, limit their effectiveness despite providing notable antiviral effects (Liang et al. 2015; Terrault et al. 2018).

As a result, interest in plant-derived compounds as alternative or complementary treatment options

has grown (Newman and Cragg 2020). Medicinal plants contain a range of different phytochemicals that can provide antioxidant and liver-protective effects through various pathways. These natural compounds often have better safety profiles than synthetic drugs (Salleret al. 2001; Singh et al. 2016). Plant secondary metabolites are particularly intriguing for treating liver disease since they can target multiple issues at once, including oxidative stress, inflammation, and viral proteins (Manikandan et al. 2017).

Euphorbia hirta L. (Euphorbiaceae), commonly known as snakeweed or asthma weed, is a herb found worldwide and has been used in traditional medicine in Africa, Asia, and the Americas to treat various conditions like digestive, skin, and respiratory issues (Kumar et al. 2010). Phytochemical studies have identified numerous bioactive compounds in this plant, including flavonoids, tannins, phenolic acids, triterpenoids, and alkaloids (Basma et al. 2011; Johnson et al. 1999). Preclinical

studies have confirmed its anti-inflammatory, antibacterial, and pain-relieving properties (Lanhers et al. 1991; Galvez et al. 1993). However, a systematic evaluation of its potential to protect the liver, especially regarding HBV-related diseases, is still lacking.

In viral hepatitis, oxidative stress plays a central role in the disease's development. The excessive production of reactive oxygen species (ROS) can overwhelm the body's natural antioxidant defenses, leading to DNA breaks, protein damage, and lipid oxidation (Sharma et al. 2012; Cichoż-Lach and Michalak 2014; Li et al. 2022; Ivanov et al. 2023). Disruption of this balance results in more liver cell damage and faster fibrotic growth. Therefore, antioxidant phytochemicals that can restore this balance are promising candidates for liver protection (Valko et al. 2007; Rehman et al. 2023). Combining in vitro antioxidant profiling with in silico molecular docking offers a thorough and efficient way to evaluate plant-derived bioactives (Mukherjee et al. 2019; Kandasamy et al. 2024). Computational docking allows for precise predictions of how well compounds will bind to relevant protein targets (Kitchen et al. 2004; Meng et al. 2011).

Objectives

This study examines the antioxidant and liver-protective properties of *E. hirta* leaves. The objectives were to build a substantial foundation of evidence supporting the use of *E. hirta* in developing plant-based liver-protective medications.

MATERIAL AND METHODS

Plant Material Procurement and Botanical Authentication

Fresh leaves of *Euphorbia hirta* L. were collected locally in Trichy. The leaves were washed under running tap water and then rinsed with distilled water to remove any contaminants.

Extract Preparation

To preserve temperature-sensitive bioactive components, the cleaned leaves were shade-dried for 14 days at room temperature. The dried leaves were ground and passed through a 40-mesh screen to obtain uniform particle size. Using a Soxhlet apparatus, 100 g of the powdered leaves was contin-

uously hot extracted with 500 mL of 99.9 percent ethanol for 72 hours at 60-65°C until the solvent in the siphon tube was clear, indicating complete extraction (Azwanida 2015). A rotary evaporator set at 45°C concentrated the extract under low pressure, and a vacuum desiccator was used to remove the solvent. The dry extract was stored in amber glass vials at 4°C until needed for experiments. Extraction efficiency was calculated as:

Yield (%) = (Mass of dried extract ÷ Mass of dried plant powder) × 100

Qualitative Phytochemical Profiling

Standard qualitative tests were performed on the ethanolic extract to identify major classes of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, glycosides, and steroids, following established methods (Harborne 1998; Trease and Evans 2002).

Quantitative Phytochemical Analysis

Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used to determine TPC (Singleton et al. 1999). In brief, 2.5 mL of Folin-Ciocalteu reagent (1:10 dilution) was mixed with 0.5 mL of 1 mg/mL extract solution. After letting it sit at room temperature for five minutes, 2 mL of 7.5 percent sodium carbonate was added. Absorbance was measured at 765 nm after 90 minutes of dark incubation. Results were expressed in milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g) using a standard curve of gallic acid.

Total Flavonoid Content (TFC)

The aluminum chloride method was used to assess TFC (Chang et al. 2002). One milliliter of 1 mg/mL extract was diluted with four milliliters of distilled water. After five minutes, the mixture received 0.3 mL of 5 percent sodium nitrite, followed by 0.3 mL of 10 percent aluminum chloride, and finally two milliliters of 1 M sodium hydroxide. The volume was adjusted to 10 mL, and absorbance was measured at 510 nm against a reagent blank. Results were reported in milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

Total Tannin Content (TTC)

The Folin-Denis method was utilised to measure TTC (Schanderl 1970). The mixture of 0.5 mL of extract, 3.75 mL of distilled water, and 0.25 mL of Folin-Denis reagent was incubated for three minutes before adding 0.5 mL of saturated sodium carbonate. Absorbance was taken at 760 nm after 90 minutes at room temperature, reporting results in milligrams of tannic acid equivalents per gram of dry extract (mg TAE/g).

In Vitro Antioxidant Activity Assessment

DPPH Free Radical Scavenging Assay (Brand-Williams et al. 1995), Hydrogen Peroxide Scavenging Assay (Ruch et al. 1989), Ferric Reducing Antioxidant Power (FRAP) Assay (Benzie and Strain 1996) and Total Antioxidant Capacity (TAC) (Prieto et al. 1999).

GC-MS Chemical Profiling

The ethanolic extract's volatile and semi-volatile chemical composition was analysed using GC-MS with an Agilent 7890B GC system connected to a 5977A mass-selective detector. The chromatographic separation used a DB-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) at a flow rate of 1 mL/min with helium as the carrier gas. The injection volume was 1 μL at a 10:1 split ratio and the injector temperature was set at 280°C. The oven program started at 60°C (holding for 2 minutes), ramping at 5°C/min to 280°C, which was held for 10 minutes. Electron ionisation (EI) mode at 70 eV was used, with source and interface temperatures at 230°C and 280°C, respectively. Mass spectra were acquired over the range of m/z 50-600. Compound identification was based on retention indices and mass spectral data compared against NIST and Wiley libraries, with relative abundance given as a percentage of peak area.

In Silico Molecular Docking Analysis

Macromolecular and Ligand Preparation

The crystallographic structure of the HBx protein was obtained from the RCSB Protein Data Bank (PDB ID: 6HT4) (Berman et al. 2000). Protein preprocessing was done in AutoDock Tools 1.5.6. This

included removing crystallographic water molecules, adding polar hydrogen atoms, and assigning Kollman partial charges before converting to PDBQT format (Morris et al. 2009). Ligand structures for compounds identified by GC-MS were retrieved from the PubChem database in SDF format. They were geometry-optimised using the MM2 force field in ChemDraw, converted to PDBQT format via Open Babel, and assigned Gasteiger charges.

Docking Protocol

Molecular docking was performed in AutoDock Vina 1.1.2 (Trott and Olson 2010). The docking grid box measured 60 × 60 × 60 Å with a spacing of 0.375 Å and was centred on the HBx active site cavity. An exhaustiveness parameter of 8 was used to ensure thorough sampling of conformations. The best binding pose for each ligand was chosen based on the most negative binding free energy value.

Binding Interaction Visualisation

Ligand-protein docked complexes were rendered and analysed in PyMOL 2.5 and Discovery Studio Visualiser 2021. Non-covalent interaction profiles were documented. This included hydrogen bonds, hydrophobic contacts, δ-δ stacking, and van der Waals forces, along with binding energies, inhibition constants, and the identities of interacting residues.

Statistical Methods

All experimental determinations were conducted in triplicate and expressed as mean ± standard deviation (SD). Statistical comparisons were made using GraphPad Prism v9.0, with one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference post hoc test. Significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction Efficiency and Preliminary Phytochemical Screening

Soxhlet extraction of *E. hirta* leaves using absolute ethanol produced a dark greenish-brown

solid with a characteristic aromatic smell. The percentage yield was 14.8 percentage (w/w). This result aligns with previously reported yields for ethanolic Euphorbia extracts, which range from 10 percentage to 18 percentage. This confirms ethanol's effectiveness as a solvent for extracting both polar and moderately polar compounds, including polyphenols, alkaloids, and glycosides (Kumar et al. 2010; Basma et al. 2011; Sulaiman et al. 2022; Rehman et al. 2023).

Qualitative phytochemical profiling of the ethanolic extract showed a wide variety of secondary metabolites (Table 1). Strongly positive reactions were observed for alkaloids, flavonoids, tannins, terpenoids, and phenols. There was moderate evidence for saponins, cardiac glycosides, and steroids. A weakly positive result was found for anthraquinone glycosides. This phytochemical profile matches reports on the species from previous studies (Lanhers et al. 1991; Johnson et al. 1999; Al-Shehri et al. 2021; Manikandan et al. 2023).

Table 1: Qualitative phytochemical screening of ethanolic extract of *E. hirtaleaves*

Phytochemical constituent	Results
Alkaloids	+++
Flavonoids	+++
Tannins	+++
Saponins	++
Terpenoids	+++
Phenols	+++
Cardiac glycosides	++
Anthraquinone glycosides	+
Steroids	++

+++ = abundantly present; ++ = moderately present; + = trace presence

Since flavonoids, tannins, and phenolic compounds are well-known mediators of antioxidant and hepatoprotective activity, their detection is especially important from a pharmacological standpoint (Nijveldt et al. 2001; Zhang et al. 2022). While

saponins and glycosides have additional immunomodulatory and cardioprotective potential (Hostettmann and Marston 1995; Singh et al. 2023), alkaloids and terpenoids provide complementary anti-inflammatory and antimicrobial qualities (Cushnie et al. 2014; Wink 2015; Elansary et al. 2024).

Quantitative Phytochemical Composition

Spectrophotometric quantification of principal phytochemical classes in the ethanolic extract is presented in Table 2.

A significant reservoir of electron-donating phenolic hydroxyl groups that can chelate pro-oxidant metal ions and neutralise free radicals is indicated by the total phenolic content of 187.4 ± 5.8 mg GAE/g (Shahidi and Ambigaipalan 2015). A rich pool of structurally diverse polyphenols known to inhibit ROS-generating enzymes and modulate oxidative stress signalling cascades is reflected in the total flavonoid content of 94.6 ± 3.2 mg QE/g (Panche et al. 2016). Given the well-established free radical scavenging, antimicrobial, and anti-inflammatory properties of condensed and hydrolysable tannins, the total tannin content of 112.3 ± 4.5 mg TAE/g further supports the antioxidant capacity (Chung et al. 1998). The concurrent abundance of these three phytochemical categories provides a mechanistic rationale for the plant's traditional hepatoprotective reputation and offers a basis for the in vitro antioxidant findings reported below.

In Vitro Antioxidant Evaluation

DPPH Radical Scavenging Activity

Throughout the tested concentration range, the ethanolic extract showed progressive, concentration-dependent DPPH radical scavenging activity (Table 3). With an IC_{50} of 42.8 ± 1.5 μ g/mL, scavenging efficiency rose from 28.4 ± 1.8 percent-

Table 2: Quantitative phytochemical composition of ethanolic extract of *E. hirtaleaves*

Parameter	Measured value	Standard
Total Phenolic Content	187.4 ± 5.8 mg GAE/g	Gallic acid
Total Flavonoid Content	94.6 ± 3.2 mg QE/g	Quercetin
Total Tannin Content	112.3 ± 4.5 mg TAE/g	Tannic acid

Values are expressed as mean $\pm$ SD ($n = 3$). GAE = gallic acid equivalents; QE = quercetin equivalents; TAE = tannic acid equivalents

Table 3: *In Vitro* antioxidant activities of ethanolic extract of *E. hirta* leaves

Concentration (µg/mL)	DPPH scavenging (%)	H ₂ O ₂ scavenging (%)	FRAP (µmol Fe ²⁺ /g)
10	28.4 ± 1.8	24.7 ± 1.5	145.3 ± 6.2
20	41.6 ± 2.1	38.9 ± 2.0	268.7 ± 8.4
40	56.8 ± 1.9	52.4 ± 1.8	412.5 ± 9.6
60	68.3 ± 2.4	65.1 ± 2.3	586.4 ± 11.3
80	79.1 ± 2.0	76.8 ± 2.1	724.8 ± 10.7
100	89.6 ± 2.3	85.4 ± 2.5	891.2 ± 12.8
IC ₅₀ (extract)	42.8 ± 1.5 µg/mL	46.3 ± 1.8 µg/mL	-
Ascorbic acid IC ₅₀	18.6 ± 0.9 µg/mL	21.4 ± 1.1 µg/mL	-

Values are expressed as mean ± SD (n = 3). IC₅₀ values represent the concentration (µg/mL) required to achieve 50% inhibition.

age at 10 µg/mL to 89.6 ± 2.3 percentage at 100 µg/mL. The extract's scavenging potency is still pharmacologically significant even though this value is higher than that of ascorbic acid (IC₅₀ = 18.6 ± 0.9 µg/mL). The ability of phenolic hydroxyl groups to transfer hydrogen atoms to DPPH radicals and reduce them to stable diamagnetic species is consistent with the concentration-response relationship (Kedare and Singh 2011).

Hydrogen Peroxide Scavenging Activity

Despite not being a radical oxidant, hydrogen peroxide is a biologically significant ROS that can pass through cellular membranes and produce extremely harmful hydroxyl radicals through Fenton-type chemistry, which can cause macromolecular oxidative damage (Halliwell and Gutteridge 2015). With an inhibition of 85.4 ± 2.5 percent at 100 µg/mL (IC₅₀ = 46.3 ± 1.8 µg/mL; ascorbic acid reference: 21.4 ± 1.1 µg/mL), the extract attenuated H₂O₂ in a dose-responsive manner. Electron donation from phenolic components to H₂O₂ is most likely the mechanistic basis, preventing the subsequent formation of hydroxyl radicals (Gulcin 2012). This activity directly relates to hepatoprotection because the liver is exposed to prolonged oxidative stress during metabolic and inflammatory processes.

Ferric Reducing Antioxidant Power (FRAP)

FRAP values increased from 145.3 ± 6.2 to 891.2 ± 12.8 µmol Fe²⁺/g over the 10–100 µg/mL range, indicating a strong concentration-dependent ferric reducing capacity in the extract (Table 3). According to Prior et al. (2005) and Apak et al. (2016), polyphenolic compounds with multiple free hydroxyl groups exhibit an electron-donating pro-

pensity that is well-positioned to reduce Fe³⁺ to Fe²⁺ and thus disrupt oxidative chain reactions.

Total Antioxidant Capacity (TAC)

A TAC of 342.7 ± 9.4 mg AAE/g was obtained from phosphomolybdenum reduction, indicating the extract's cumulative electron-transfer antioxidant capacity. This high value is consistent with the concurrent abundance of phenolics, flavonoids, and tannins supporting the multimechanistic antioxidant activity shown by the DPPH, H₂O₂, and FRAP assays (Prieto et al. 1999). A complete picture of the extract's antioxidant potential is provided by the complementary nature of these four antioxidant platforms, each of which probes unique mechanistic aspects of radical neutralisation and reducing power. Importantly, given the critical role of oxidative stress in HBV-associated hepatocellular injury, this strong antioxidant activity directly supports hepatoprotective function (Cichoz-Lach and Michalak 2014).

GC-MS Chemical Composition Analysis

GC-MS profiling of the ethanolic *E. hirta* leaf extract resolved 20 discrete chromatographic peaks, collectively accounting for 92.8 percent of total ion current (Table 4). Identified constituents spanned six chemical classes for saturated and unsaturated fatty acids and their methyl esters, diterpene alcohols, triterpene hydrocarbons, phytosterols, pentacyclic triterpenoids, and polyphenolic flavonoids and phenolic acids.

Twenty compounds were identified in plant extract by GC-MS analysis. The active principles with their retention time, molecular formula, molec-

Table 4: Chemical composition of ethanolic extract of *E. hirta* leaves determined by GC-MS analysis

Peak	RT (min)	Area %	Compound
1	5.287	0.48	2(3h)-Furanone, Dihydro-
2	6.092	2.20	2-Furancarboxaldehyde, 5-Methyl-
3	6.315	1.00	2,4-Dihydroxy-2,5-Dimethyl-3 (2H)-Fural-3-one
4	7.493	0.38	Pentanoic Acid, 4-Oxo-
5	7.992	4.29	Cyclopentane, 1-Acetyl-1,2-Epoxy
6	8.148	1.47	2-Heptanone, 3-Methyl-
7	8.958	0.41	2-Acetyl-2-Hydroxy-, Gamma-, Butyro
8	9.123	9.65	4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Me
9	10.405	54.02	Furan, 2,3-Dihydro-4-(1-Methylpropyl)-, (S)-
10	12.690	3.18	DL-Proline, 5-Oxo-, Methyl Ester
11	13.705	0.26	3-O-Benzyl-D-Glucose
12	14.414	0.43	D-Gluco-Heptulosan
13	15.817	18.35	Asarone
14	16.553	0.45	1-Allyl-2,4,5-Trimethoxy-Benzene
15	17.399	0.28	Tetracosanoic Acid, 23-Oxo-, Methyl
16	18.744	0.39	2-Tridecenal, (Z)-
17	19.194	0.28	Octadecanoic Acid, Methyl Ester
18	19.574	1.64	9-Octadecenoic Acid (Z)-
19	19.757	0.33	1,2-Benzenedicarboxylic Acid, Dibut
20	20.861	0.52	1-Octadecanol

ular weight and concentration (%) are presented in Table 5 and Figure 1.

The prevailing compounds are 2-heptanone, 3-methyl, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, asarone, 1-allyl-2,4,5-trimethoxybenzene, tetracosanoic acid, 23-oxo-methyl, 2-tridecenal, (Z), octadecanoic acid methyl ester, 9-octadecenoic acid (Z), 1,2-benzenedicarboxylic acid and the compounds such as benzene dicarboxylic acid and heptanone, which contains the anti-microbial activity, octadecanoic acid, 4H-pyran 4-one, which contains the anti-inflammatory activity. Octadecenoic acid contains the anti-hypertensive and hypercholesterolemic activity (Dr. Duke's phytochemical and ethano-botanical database).

The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners (Manikandan et al. 2017). However isolation of individual phytochemical constituents and subjecting its biological activity will definitely give fruitful results. Both plant leaves extracts contain octadecanoic acid as a component. It may be the reason for the various medicinal activities among these that the further study is planned to find hepatoprotective activity by binding properties of the mediator called HBx protein of hepatitis B virus with octadecanoic acid. 1-octadecanol were found in this plant.

The compositional diversity of the extract spanning lipophilic terpenoids and sterols alongside

Table 5: Docking results of phytoconstituents against the Hepatitis-B virus protein

Protein	Compounds
Hepatitis-B	virus X protein
	1. PentanoicAcid:CCCCC(=O)O
	2.Cyclopentane:C1CCCC1
	3.2-Heptanone:CCCCC(=O)C
	4.TetracosanoicAcid:CCCCCCCCCCCCCCCCCCCCCCCC(=O)O
	5.Asarone:COC1=CC=C(C=C1OC)C=C
	6.OctadecanoicAcid:CCCCCCCCCCCCCCCCCCCC(=O)O
	7.1,2-BenzenedicarboxylicAcid:OC(=O)C1=CC=CC=C1C(=O)O
	8.1-Octadecanol:CCCCCCCCCCCCCCCCCO

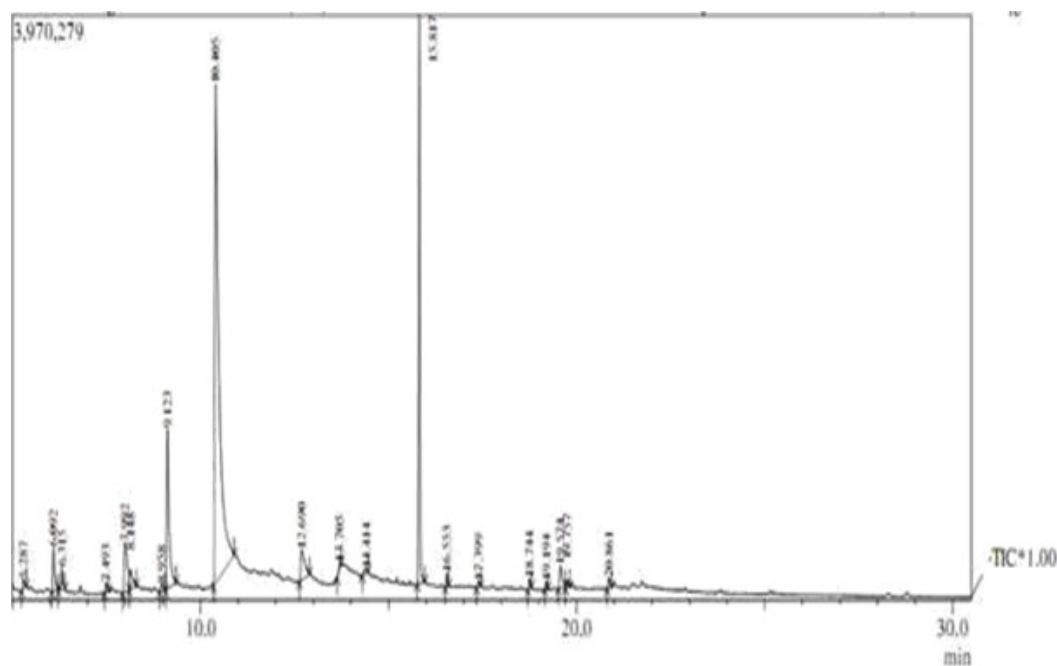


Fig. 1. GC-MS results of Ethanolic extract of *E. hirta*

hydrophilic polyphenols supports a multi-mechanistic hepatoprotective model in which compounds with differing physicochemical properties act synergistically across aqueous and lipid compartments.

Molecular Docking Analysis

Eight phytocompounds derived from *E. hirta* were tested for their ability to bind to the Hepatitis B virus X protein (HBx) using molecular docking simulations. Binding affinities were calculated us-

ing AutoDock Vina and expressed as Vina scores (kcal/mol), where stronger ligand-receptor interactions are indicated by more negative values. The macromolecular target was the three-dimensional crystal structure of HBx (PDB ID: 7VIF). Before docking, all ligands underwent energy minimisation, and the ideal docking grid was identified using calculated cavity volumes and centre coordinates. Figure 2 represent the structure of HBX-1 protein. The molecular interactions are shown in the Figures 3 - 10.

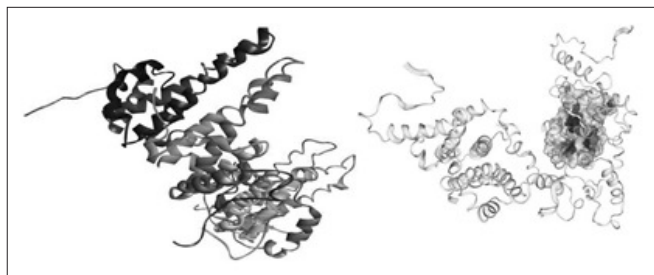


Fig. 2. 3D structure and Actisite of HBX1

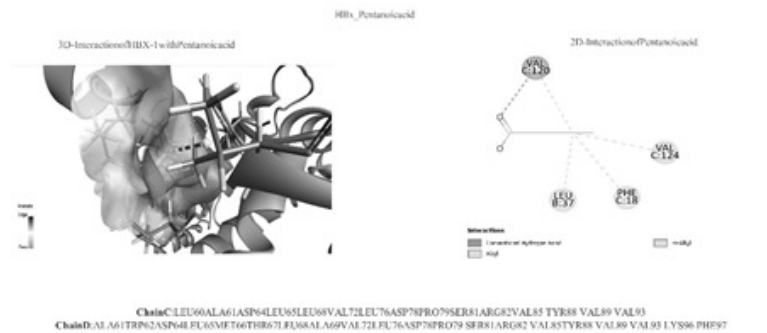


Figure 3: HHX-Pentanoicacid Interaction

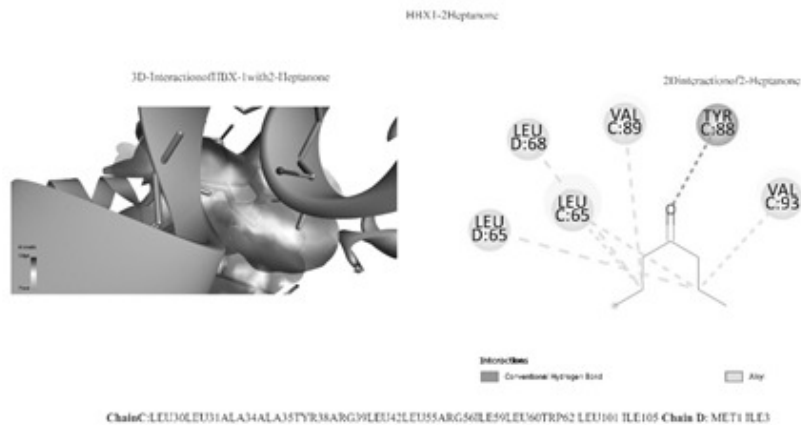
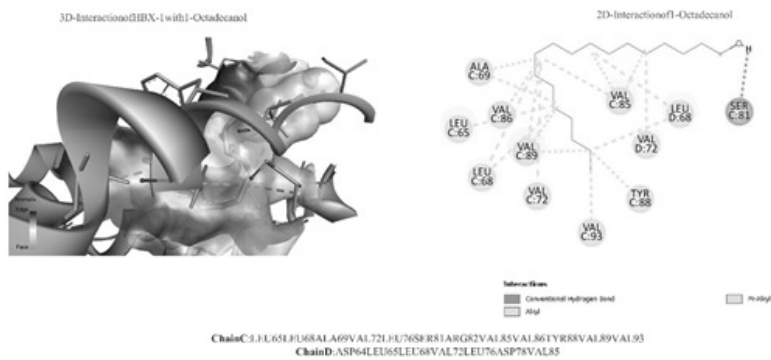


Figure 5: HHX-1-2-Heptanone Interaction

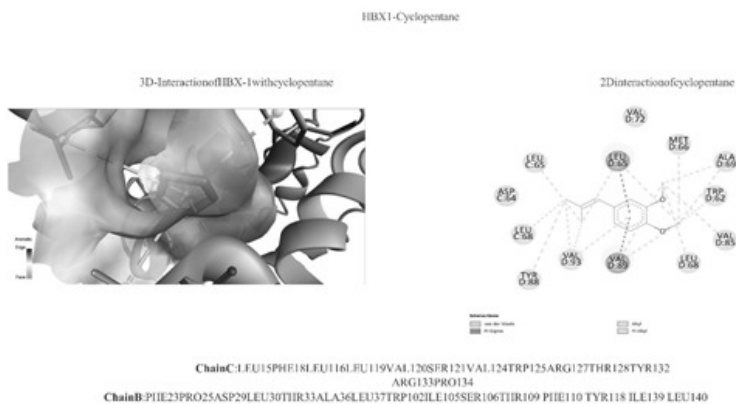
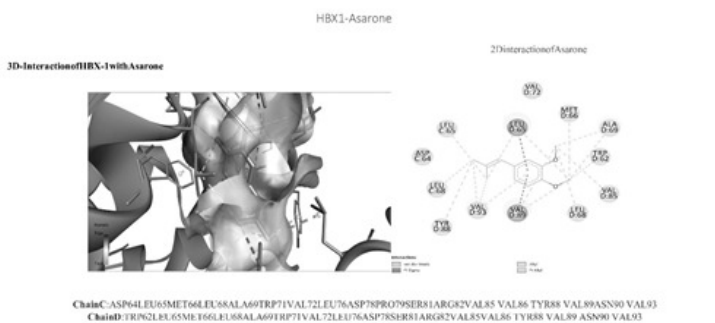


Figure 8: HBX1-Cyclopentane Interaction

HBX1-Cyclopentane

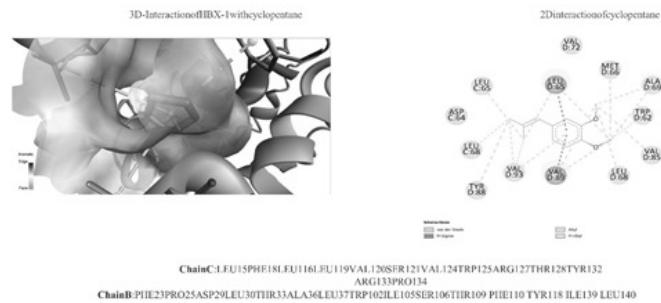


Figure 8: HBX1-Cyclopentane Interaction

HBX1-Octadecanoic acid

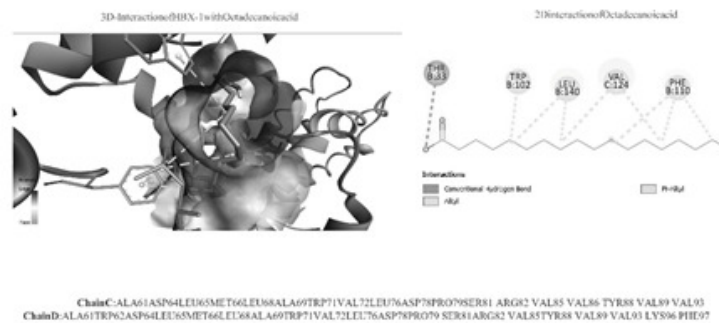


Figure 9: HBX1-Octadecanoic acid Interactions

HBX1_Tetracosanoic Acid

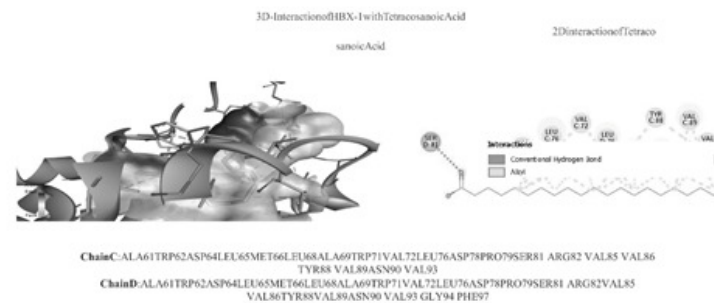


Figure 10: HBX1_Tetracosanoic Acid

Tetracosanoic acid had the highest binding affinity of the eight compounds screened, with a Vina score of -7.6 kcal/mol (Rank 1), followed by 1-Octadecanol at -6.6 kcal/mol (Rank 3) and Octadecanoic acid at -6.8 kcal/mol (Rank 2). When compared to the other ligands, these long-chain fatty acids and fatty alcohol showed noticeably better binding. The phenylpropanoid component asarone had a Vina score of -6.5 kcal/mol (Rank 4). Phthalic acid, also known as 1,2-benzenedicarboxylic acid, scored -6.0 kcal/mol (Rank 5). The compounds with lower binding affinities were Pentanoic acid (-4.5 kcal/mol), Cyclopentane (-4.6 kcal/mol), and 2-Heptanone (-4.8 kcal/mol). The full docking parameters for each of the eight ligand-protein complexes are compiled in Table 5. Summary of docking score are given in Table 6.

Binding Interactions of Top-Ranked Compounds

Tetracosanoic Acid (Rank 1; Vina Score: -7.6 kcal/mol)

Among all the substances tested, tetracosanoic acid (lignoceric acid) showed the highest binding

affinity. Interactions with residues of Chains C and D (ALA61, TRP62, ASP64, LEU65, MET66, LEU68, ALA69, TRP71, VAL72, LEU76, ASP78, PRO79, SER81, ARG82, VAL85, VAL86, TYR88, VAL89, ASN90, VAL93, GLY94, PHE97). Tetracosanoic acid occupies a broad cavity volume of 4541 Å³, which is consistent with its long acyl chain architecture, according to the extensive involvement of hydrophobic and polar residues spanning both chains. The participation of key residues such as ARG82, TRP71, and TYR88 in the active binding pocket implies strong hydrophobic contacts and possible hydrogen bonding interactions that collectively stabilise the complex.

Octadecanoic Acid (Rank 2; Vina Score: -6.8 kcal/mol)

Chain C (ALA61, TRP62, ASP64, LEU65, MET66, LEU68, ALA69, TRP71, VAL72, LEU76, ASP78, PRO79, SER81, ARG82, VAL85, VAL86, TYR88, VAL89, VAL93) and Chain D (ALA61, TRP62, ASP64, LEU65, MET66, LEU68, PRO79, SER81, ARG82, VAL85, TYR88, PHE97). Octadecanoic acid interacts with the same cavity (4541

Table 5: Docking results of phytoconstituents against the Hepatitis-B virus protein

Protein	Compounds
Hepatitis-B	virus X protein 1. PentanoicAcid:CCCCC(=O)O 2.Cyclopentane:C1CCCC1 3.2-Heptanone:CCCCC(=O)C 4.TetracosanoicAcid:CCCCCCCCCCCCCCCCCCCCCCCC(=O)O 5.Asarone:COC1=CC=C(C=C1OC)C=C 6.OctadecanoicAcid:CCCCCCCCCCCCCCCCCCCC(=O)O 7.1,2-BenzenedicarboxylicAcid:OC(=O)C1=CC=CC=C1C(=O)O 8.1-Octadecanol:CCCCCCCCCCCCCCCCCO

Table 6: Summary of molecular docking interactions of HBx protein with phytocompounds from *E. hirta*

Name of ligand	Ranking	Vina score (kcal/mol)	Cavity volume (Å ³)	Centre (x,y,z)	Docking box size
Tetracosanoic acid	1	"7.6	4541	221, 89, 113	30, 30, 30
Octadecanoic acid	2	"6.8	4541	221, 89, 113	32, 26, 35
1-Octadecanol	3	"6.6	4541	221, 89, 113	32, 23, 35
Asarone	4	"6.5	4541	221, 89, 113	32, 25, 35
1,2-Benzenedicarboxylic acid	5	"6.0	951	198, 89, 139	17, 17, 32
2-Heptanone	6	"4.8	740	216, 74, 118	17, 17, 17
Cyclopentane	7	"4.6	951	198, 89, 139	17, 17, 32
Pentanoic acid	8	"4.5	4541	221, 89, 113	32, 25, 35

Å³ = cubic angstroms; kcal/mol = kilocalories per mole

Å³) as tetracosanoic acid, and the docking pose shows significant hydrophobic surface complementarity along the fatty acid chain. Potential polar and electrostatic contributions to complex stabilisation are further implied by the presence of ASP64, ARG82, and LYS96 in the binding interface.

1-Octadecanol (Rank 3; Vina Score: -6.6 kcal/mol)

1-Octadecanol, which is the fatty alcohol form of stearic acid, had interactions with Chain C residues LEU65, LEU68, ALA69, VAL72, LEU76, SER81, ARG82, VAL85, VAL86, TYR88, VAL89, VAL93 and Chain D residues ASP64, LEU65, LEU68, VAL72, LEU76, ASP78, VAL85. The hydroxyl group at the terminus of this compound can form hydrogen bonds with polar residues SER81 and ARG82, which explains its high binding score. The slightly reduced number of residues is probably due to minor adjustment in position in the same binding cavity.

Asarone (Rank 4; Vina Score: -6.5 kcal/mol)

Asarone, a phenylpropanoid ether compound known to be linked with the pharmacological properties of *Acorus calamus*, showed promising binding affinities. The residues in contact with the compound were Chain C (ASP64, LEU65, MET66, LEU68, ALA69, TRP71, VAL72, LEU76, ASP78, PRO79, SER81, ARG82, VAL85, VAL86, TYR88, VAL89, ASN90, VAL93) and Chain D (TRP62, LEU65, MET66, LEU68, ALA69, TRP71, VAL72, LEU76, ASP78, SER81, ARG82, VAL85, VAL86, TYR88, VAL89, ASN90, VAL93). Interestingly, some binding residues were common with the top-ranking fatty acids, like TRP71, ARG82, and TYR88.

1,2-Benzenedicarboxylic Acid (Rank 5; Vina Score: -6.0 kcal/mol)

Phthalic acid had its own binding pocket with a reduced cavity volume of 951 Å³ and different coordinates for the centre (198, 89, 139). This suggests that it binds to an alternative site on the HBx protein. The residues that contribute to its binding are VAL120, SER121, PHE122, VAL124, TRP125, ILE126, THR128, PRO129, TYR132, ARG133, PRO134 from Chain C and PHE23, PRO25, THR33, LEU37, TYR38, TRP102, ILE105, SER106, THR109,

PHE110, TYR118, ILE139, LEU140, SER141, THR142 from Chain B. The aromatic dicarboxylic acid structure can accommodate δ -stacking with TRP125 and TYR132 residues and hydrogen bonding with THR128 and SER121 residues at this secondary binding site.

2-Heptanone, Cyclopentane, and Pentanoic Acid (Ranks 6-8)

The remaining three compounds demonstrated moderate to low binding affinities. 2-Heptanone bound to Chain C residues LEU30, LEU31, ALA34, ALA35, TYR38, ARG39, LEU42, LEU55, ARG56, ILE59, LEU60, TRP62, LEU101, and ILE105, and Chain D residues MET1 and ILE3 in a relatively compact space of 740 Å³. Cyclopentane is bound to the same 951 Å³ space containing phthalic acid with a moderate binding affinity of -4.6 kcal/mol. This ligand also interacted with Chain C residues LEU15, PHE18, LEU116, LEU119, VAL120, SER121, VAL124, TRP125, ARG127, THR128, TYR132, ARG133, PRO134 and Chain B residues. Finally, pentanoic acid is bound to the primary 4541 Å³ space with a low binding affinity of -4.5 kcal/mol. This ligand demonstrated poor interaction with the protein due to the short length of the ligand's carbon chain. It interacted with Chain C residues LEU60, ALA61, ASP64, LEU65, LEU68, VAL72, LEU76, ASP78, PRO79, SER81.

In the present investigation, structure-based molecular docking studies were conducted to investigate the inhibitory potential of eight different phytoconstituents isolated from *E. hirta* against the Hepatitis B virus X protein (HBx), which is a multifunctional non-structural protein essential for viral replication, transcriptional transactivation, and oncogenic transformation of hepatocytes (Andrisani and Barnabas 1999; Feitelson and Lee 2007). HBx interferes with host cell processes such as the ubiquitin-proteasome pathway, DNA repair processes, and apoptosis. This protein is considered a major target in the development of therapeutic strategies to treat chronic hepatitis B infection and HBx-positive hepatocellular carcinoma (HCC) (Lian et al. 2019; Tang et al. 2023). Recent studies have further reinforced the therapeutic importance of HBx, identifying it as a promising target for achieving a functional cure of chronic HBV infection because HBx regulates viral transcription and promotes degradation of host restriction factors such

as the Smc5/6 complex (Kumar 2025; Giriand Kumar 2026). Structural studies published recently have also provided new insights into the interaction of HBx with DDB1, further supporting the structural and mechanistic relevance of HBx as a druggable viral target (Tanaka et al. 2025).

Among all compounds tested, tetracosanoic acid, or lignoceric acid, had the highest binding affinity at -7.6 kcal/mol. This is consistent with the known binding properties of saturated fatty acids, which have been shown to have extensive hydrophobic binding pockets. In particular, saturated fatty acids within this range have been shown to play a role in viral replication complexes. These compounds have been shown to have a high binding affinity to the hydrophobic regions of non-structural viral proteins. This has been shown through various in silico studies (Vijayakumar et al. 2021; Shaik et al. 2022). Recent phytochemical investigations of *E. hirta* have continued to demonstrate the presence of fatty acids and other lipid-like constituents, supporting the relevance of long-chain lipid compounds within the phytochemical profile of this plant (Kaba et al. 2025; Mbwaile et al. 2025). The impressive role that ARG82, TRP71, and TYR88 play among all ligands with high binding affinities indicates that these residues play a critical role in HBx binding. These residues have been shown to play a role in guanidinium-mediated hydrogen bonding or salt-bridge formation. Tryptophan and tyrosine have been shown to play a role in hydrophobic interactions that are critical to ligand binding.

Octadecanoic acid (-6.8 kcal/mol) and 1-Octadecanol (-6.6 kcal/mol) also utilised the main 4541 Å³ cavity in a similar fashion and also showed a high overlap in their repertoire of binding residues, as observed in tetracosanoic acid. This similarity in structure is not surprising, as the carbon chain length in both compounds is similar. The small decrease in binding energy from tetracosanoic to octadecanoic acid might be due to the four methylene groups in lignoceric acid, which provide a better opportunity for increased van der Waals interactions in the elongated hydrophobic channel. Previous studies on the interactions between fatty acids and viral capsid proteins have established a positive correlation between fatty acid chain length and binding free energy, up to a certain limit, after which steric hindrance also plays a role in reducing the complementarity of the interactions (Bhatt

et al. 2020). The presence of a hydroxyl group in 1-Octadecanol provides a hydrogen bonding site not seen in the fatty acids, which might be a reason for its retaining a high level of binding affinity even in the absence of a carboxylate functional group. Recent computational phytochemical studies similarly emphasize that hydrophobic contacts, hydrogen bonding, ligand size, and the physicochemical characteristics of natural compounds can substantially influence predicted protein–ligand interactions and their ranking during virtual screening (Ibrahim and Ayipo 2025; Ancuceanu et al. 2025).

Asarone, which has been most extensively investigated in terms of pharmacological activities, had a calculated Vina score of -6.5 kcal/mol, which again corroborated previous findings on the antiviral and hepatoprotective potentials of this compound. Asarone has been shown to possess antiviral, anti-inflammatory, and hepatoprotective activities, as reflected by the inhibition of lipid peroxidation, production of inflammatory cytokines, and markers of liver cell injury (Gilani et al. 2006; Mehta et al. 2013). In terms of structural analysis, the trimethoxybenzene ring in the asarone molecule is quite suitable to interact with TRP71 and TYR88 by δ -stacking, while the vinyl side chain is likely to contribute to hydrophobic interactions with the viral protease enzyme. These results are in partial agreement with those of Rai et al. (2021), which showed asarone to be a potent inhibitor of viral proteases by virtue of identical binding modes. The presence of ASN90 in the interfaces of Chain C and Chain D in the asarone complex is likely to contribute to hydrogen bonding by the side-chain amide, which may contribute to the selectivity of the binding. The continued investigation of plant-derived phenylpropanoids and other secondary metabolites using molecular docking and complementary computational approaches supports the potential of such compounds as lead structures for antiviral drug discovery, although computational predictions require experimental confirmation (Ancuceanu et al. 2025; Taha and Daoud 2026).

Phthalic acid (1,2-benzenedicarboxylic acid) had a remarkably different binding profile that involved a secondary binding cavity of 951 Å³ at coordinates (198, 89, 139), with a score of -6.0 kcal/mol. The identification of a secondary binding site is of interest from a mechanistic perspective because allosteric inhibition has been shown to be a viable strategy for targeting HBx, owing to the presence

of intrinsically disordered regions of HBx and the complex interaction network of multiple domains (Kang and Shen 2023). The importance of identifying alternative or non-canonical binding sites has gained further attention in contemporary structure-based drug discovery, particularly for targets exhibiting conformational flexibility and complex protein–protein interactions (Giri and Kumar 2026; Altharawi and Alqahtani 2026). The aromatic ring of phthalic acid enables stacking interactions with TRP125 and TYR132 residues, whereas both carboxylate groups enable hydrogen bond formation with polar residues THR128 and SER121. Dicarboxylic aromatic compounds have been studied as a potential scaffold for allosteric antiviral agents. The current study provides a rationale for the potential of phthalic acid derivatives to be further explored for their antiviral activity (Martelli et al. 2014).

The low-affinity ligands 2-heptanone (−4.8 kcal/mol), cyclopentane (−4.6 kcal/mol), and pentanoic acid (−4.5 kcal/mol) possessed binding scores below the empirical cutoff of −5.0 kcal/mol typically employed to discriminate between biologically relevant binders and non-specific interactions in virtual screening exercises (Trott and Olson 2010). The decreased affinity of pentanoic acid compared to octadecanoic acid, which possess the same carboxylate-containing pharmacophore group, underscores the significant role played by hydrophobic chain length in facilitating productive binding to the large HBx ligand-binding cavity. Cyclopentane lacks any functional groups and must rely on van der Waals interactions alone to bind to the receptor. This is clearly insufficient to facilitate high-affinity binding to such a large ligand-binding cavity. These data are in keeping with general SAR considerations applicable to fatty acid analogs in studies of enzyme inhibition (Dewick 2009). Recent reviews of computer-aided drug discovery have emphasized that docking scores should primarily be interpreted as comparative computational indicators rather than definitive measures of experimental binding affinity, particularly when diverse chemical classes are compared (Gogoi et al. 2026; Altharawi et al. 2026).

From a broader therapeutic point of view, the prevalence of long-chain fatty acids and phenylpropanoids asarone among the top-ranked compounds places *E. hirta* as a potential source for developing antiviral phytochemicals against HBx. Several studies have demonstrated the antiviral

potential of *E. hirta* extracts against enveloped viruses; however, HBx targeting molecular docking analysis has not been reported for this plant previously (Negi et al. 2011; Rajput et al. 2014). The current study adds novel mechanistic evidence to existing ethnopharmacological literature on this plant. Recent investigations continue to demonstrate the chemical diversity and pharmacological potential of *E. hirta*, including the identification of fatty acids, terpenoids, coumarin-related compounds, and other bioactive constituents by chromatographic and phytochemical approaches (Kaba et al. 2025; Mbale et al. 2025; Costa et al. 2025). Furthermore, recent computational studies of *E. hirta* phytochemicals against viral targets have demonstrated that compounds from this plant can exhibit favorable predicted interactions with viral proteins, providing additional support for the use of *E. hirta* as a source of antiviral lead molecules (Cayona et al. 2022).

It is, however, important to recognise the limitations in the docking results, which may not serve as the sole determinant in establishing biological activity. The score, which is a measure of binding free energy, is an approximation in static receptor conditions, which does not include protein flexibility, solvation, or off-target effects (Pagadala et al. 2017). Recent computational drug-discovery literature similarly emphasizes that conventional docking can be limited by receptor rigidity, scoring-function approximations, incomplete treatment of solvation, and the conformational flexibility of protein–ligand systems (Ancuceanu et al. 2025; Altharawi et al. 2026). Future studies should involve the use of MD simulation to validate the stability of the complex over time. In addition, binding free energy calculation, either by MM-GBSA or MM-PBSA, would be important in establishing the ranking order (Genheden and Ryde 2015). Recent studies integrating molecular docking, molecular dynamics, MM/PBSA or MM/GBSA calculations, and ADMET prediction demonstrate the value of combining complementary computational approaches to improve confidence in the prioritization of natural-product candidates (Ibrahim and Ayipo 2025; Sindi et al. 2025). Moreover, pharmacokinetic analysis (ADMET) by computational models like SwissADME and pkCSM would be important in establishing the drug-likeness of the top candidates, which would be important in future experimental validation. Recent reviews spe-

cifically highlight ADME prediction as an important component of natural-product drug discovery because promising phytochemicals can exhibit limitations related to absorption, distribution, metabolism, excretion, solubility, and toxicity despite favorable docking scores (Ancuceanu et al. 2025). The in silico study, however, offers a reproducible, hypothesis-driven approach to the advancement of *E. hirta* phytochemicals in the discovery of anti-HBV drugs.

CONCLUSION

This research offers a scientific rationale to validate the traditional use of *E. hirta* leaves in the prevention and treatment of liver and hepatic disorders. The ethanolic extract, which is rich in phytochemicals, has shown substantial antioxidant activities, which have been mechanistically validated in this study. The GC-MS analysis has revealed the presence of 20 bioactive constituents, which have shown substantial antioxidant activities in vitro, indicating a multi-targeted mechanism of action in the prevention and treatment of liver and hepatic disorders. Several limitations, which are inherent in this study, need to be addressed. In vitro antioxidant activities, although mechanistically validated, may not accurately reflect the in vivo activities, where metabolism, pharmacokinetics, and immunological responses may influence the outcome. The substantial antioxidant activities, which have been supported by in silico studies on the interaction with the HBx protein, indicate the potential utility of this plant in the prevention and treatment of viral hepatitis and liver and hepatic disorders. The therapeutic utility, however, awaits in vivo and in vitro substantiation.

RECOMMENDATIONS

Euphorbia hirta leaves, which have shown substantial antioxidant activities, are botanically and pharmacologically validated to be developed into a potential hepato-protective nutraceutical/phytopharmaceutical agent in near future.

CONFLICT OF INTEREST

The authors affirm that no financial or personal conflicts of interest exist that could have influ-

enced the design, conduct, interpretation, or publication of this research.

ETHICAL STATEMENT

This investigation involved exclusively plant-derived materials and in vitro/in silico experimental approaches. No human participants or vertebrate animals were included in the study. Plant material collection conformed to applicable institutional guidelines and local regulatory requirements.

REFERENCES

- Al-Shehri S, Malik JA, Al-Harbi NO 2021. Phytochemical characterization and biological activities of *Euphorbia hirta*: A review. *Molecules*, 26(21): 6503. <https://doi.org/10.3390/molecules26216503>
- Altharawi A, Alqahtani SM 2026. Integrative computational chemistry approaches in modern drug discovery: advances in docking, pharmacophore modeling, molecular dynamics, and virtual screening. *Pharmaceutics*, 18(5): 565. doi:10.3390/pharmaceutics18050565.
- Ancuceanu R, Lascu BE, Drăgănescu D 2025. In silico ADME methods used in the evaluation of natural products. *Pharmaceutics*, 17(8):1002. doi:10.3390/pharmaceutics17081002
- Andrisani OM, Barnabas S 1999. The transcriptional function of the hepatitis B virus X protein and its role in hepatocarcinogenesis. *International Journal of Oncology*, 15(2): 373-379. <https://doi.org/10.3892/ijo.15.2.373>
- Apak R, Özyürek M, Güçlü K, Çapanoğlu E 2016. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. *Journal of Agricultural and Food Chemistry*, 64(5): 997-1027. <https://doi.org/10.1021/acs.jafc.5b04739>
- Azwanida NN 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants*, 4(196): 1-6.
- Basma AA, Zakaria Z, Latha LY, Sasidharan S 2011. Antioxidant activity and phytochemical screening of the methanol extracts of *Euphorbia hirta* L. *Asian Pacific Journal of Tropical Medicine*, 4(5): 386-390.
- Benzie IF, Strain JJ 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, 239(1): 70-76.
- Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE 2000. The Protein Data Bank. *Nucleic Acids Research*, 28(1): 235-242.
- Bhatt S, Shome A, Gaikwad SM, Bhattacharyya D 2020. In silico and in vitro evaluation of fatty acid-derived compounds as inhibitors of viral surface proteins. *Journal of Biomolecular Structure and Dynamics*, 38(14): 4189-4201. <https://doi.org/10.1080/07391102.2019.1678661>
- Bouchard MJ, Schneider RJ 2004. The enigmatic X gene of hepatitis B virus. *Journal of Virology*, 78(23): 12725-12734.

- Brand-Williams W, Cuvelier ME, Berset C 1995. Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28(1): 25-30.
- Chang CC, Yang MH, Wen HM, Chern JC 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3): 178-182.
- Chen W, Lin C, Wang L, Yu Z, Xu Y, Zhang M 2025. Hepatitis B virus X protein promotes the progression and immune escape of hepatocellular carcinoma by activating KLF16-C12orf49-PD-L1 axis. *Oncogene*, 44: 4747-4762. doi:10.1038/s41388-025-03625-4
- Chen Z, Hu R, Ye H, Chen Q, Liu Y, Chen Y 2026. Innate immune recognition and evasion strategies of hepatitis B virus: From DNA to RNA and viral proteins. *Front Immunol*, 17: 1741620. doi:10.3389/fimmu.1741620
- Chung KT, Wong TY, Wei CI, Huang YW, Lin Y 1998. Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition*, 38(6): 421-464.
- Cichoż-Lach H, Michalak A 2014. Oxidative stress as a crucial factor in liver diseases. *World Journal of Gastroenterology*, 20(25): 8082-8091.
- Costa JA da, Marinho AO de, Alves RR de V, Barros MC de, Vila Nova IC, Oliveira SA de 2025. Phytochemical profile, toxicological screening, antitumor activity, and immunomodulatory response of saline extract from *Euphorbia hirta* L. leaves. *Molecules*, 30(15): 3105. doi:10.3390/molecules30153105
- Cushnie TP, Cushnie B, Lamb AJ 2014. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents*, 44(5): 377-386.
- Dewick PM 2009. *Medicinal Natural Products: A Biosynthetic Approach*. 3rd Edition. Chichester, West Sussex, UK: John Wiley and Sons.
- Elansary HO, Szopa A, Kubica P, Al-Mana FA, Ekiert H 2024. Terpenoids as promising anti-inflammatory and antimicrobial agents: Recent advances and therapeutic prospects. *Plants*, 13(2): 214. https://doi.org/10.3390/plants13020214
- Feitelson MA, Lee J 2007. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. *Cancer Letters*, 252(2): 157-170. https://doi.org/10.1016/j.canlet.2006.11.010
- Galvez J, Zarzuelo A, Crespo ME, Lorente MD, Ocete MA, Jiménez J 1993. Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. *Planta Medica*, 59(4): 333-336.
- Genheden S, Ryde U 2015. The MM/PSA and MM/GBSA methods to estimate ligand-binding affinities. *Expert Opinion on Drug Discovery*, 10(5): 449-461. https://doi.org/10.1517/17460441.2015.1032936
- Gilani AH, Shah AJ, Ahmad M, Shaheen F 2006. Antispasmodic effect of *Acorus calamus* Linn. is mediated through calcium channel blockade. *Phytotherapy Research*, 20(12): 1080-1084. https://doi.org/10.1002/pGiri S, Kumar V 2026. Hepatitis B virus X protein-targeted therapeutic strategies toward a functional cure for chronic hepatitis B infection: A review. *J Clin Transl Res*, 12(1): 22-38. doi:10.36922/JCTR025410069
- Gogoi PP, Singha B, Longkumer P, Boruah N, Sinha UB 2026. Classical docking to machine learning based docking: molecular docking in drug discovery. *Curr Comput Aided Drug Des.* doi:10.2174/0115680266424314251204071847
- Gulcin Y 2012. Antioxidant activity of food constituents: An overview. *Archives of Toxicology*, 86(3): 345-391.
- Ivanov AV, Valuev-Elliston VT, Ivanova ON, Kochetkov SN, Starodubova ES 2023. Oxidative stress during viral hepatitis and antiviral therapy: Mechanisms and clinical implications. *International Journal of Molecular Sciences*, 24(3): 2458. https://doi.org/10.3390/ijms24032458
- Halliwell B, Gutteridge JMC 2015. *Free Radicals in Biology and Medicine*. 5th Edition. Oxford, United Kingdom: Oxford University Press.
- Harborne JB 1998. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd Edition. London, United Kingdom: Chapman and Hall.
- Hostettmann K, Marston A 1995. *Saponins*. Cambridge, United Kingdom: Cambridge University Press.
- Ibrahim SO, Ayipo YO, Lukman HY, Abubakar FA, Na'Allah A, et al. 2025. De novo in silico screening of natural products for antidiabetic drug discovery: ADMET profiling, molecular docking, and molecular dynamics simulations. *In Silico Pharmacol*, 13(1): 29. doi:10.1007/s40203-025-00320-w.
- Johnson PB, Abdurahman EM, Tiam EA, Abdu-Aguye I, Hussaini IM 1999. *Euphorbia hirta* leaf extracts increase urine output and electrolytes in rats. *Journal of Ethnopharmacology*, 65(1): 63-69.
- Kaba S, Yogeshwari M, Malipatil M 2025. Preliminary chemical investigation and antimicrobial activity of whole plant of *Euphorbia hirta* Linn. *Res J Pharmacogn Phytochem*, 17(3): 193-196. doi:10.52711/0975-4385.2025.00031
- Kandasamy S, Almaghaslah D, Thangavelu L 2024. Integrated in vitro and in silico approaches for evaluating phytochemicals as therapeutic agents: Recent advances and future perspectives. *Journal of Biomolecular Structure and Dynamics*, 42(2): 915-930. https://doi.org/10.1080/07391102.2023.2174567
- Kang D, Shen Q 2023. Allosteric modulation of intrinsically disordered viral proteins: Emerging opportunities in antiviral drug discovery. *Drug Discovery Today*, 28(4): 103527. https://doi.org/10.1016/j.drudis.2023.103527
- Kedare SB, Singh RP 2011. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4): 412-422.
- Kitchen DB, Decornez H, Furr JR, Bajorath J 2004. Docking and scoring in virtual screening for drug discovery: Methods and applications. *Nature Reviews Drug Discovery*, 3(11): 935-949.
- Kumar S, Malhotra R, Kumar D 2010. *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharmacognosy Reviews*, 4(7): 58-61.
- Kumar V 2025. HBx protein as a therapeutic target for functional cure of hepatitis B virus infection. *Virology*, 604: 110438. doi:10.1016/j.virol.2025.110438
- Lanthers MC, Fleurentin J, Mortier F, Vinche A, Younos C 1991. Anti-inflammatory and analgesic effects of an aqueous extract of *Harpagophytum procumbens*. *Planta Medica*, 58(2): 117-123.
- Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y 2022. The role of oxidative stress and antioxidants in liver diseases. *International Journal of Molecular Sciences*, 23(9): 4875. https://doi.org/10.3390/ijms23094875

- Lian J, Lan T, Ye X, Fan L, Xu Y, Yin F 2019. The role of HBx in HBV-related hepatocellular carcinogenesis: Recent advances and future prospects. *Frontiers in Oncology*, 9: 737. <https://doi.org/10.3389/fonc.2019.00737>
- Liang TJ, Block TM, McMahon BJ, Ghany MG, Urban S, Guo JT 2015. Present and future therapies of hepatitis B: From discovery to cure. *Hepatology*, 62(6): 1893-1908.
- Manikandan R, Anand AVijaya, Sampathkumar P, Manoharan N 2017. Protective effect of Psidium guajava leaf ethanolic extract against streptozotocin-induced diabetes and lipidosis in rats. *Indian Journal of Animal Research*, 52(8): 1198-1205.
- Manikandan R, Balamuralikrishnan B, Boro A, Karthika P, Arun M, Velayuthaprabhu S, Malaisamy A, Rengarajan RL, Anand AV 2023. Impact on cardioprotective effect of Psidium guajava leaves extract in streptozotocin-induced Wistar mice with molecular in silico analysis. *Quality Assurance and Safety of Crops & Foods*, 15(2):209-221.
- Martelli G, Giacomini D, Brancale A 2014. Dicarboxylic acid derivatives as allosteric inhibitors of viral protein assemblies. *Antiviral Chemistry and Chemotherapy*, 23(6): 255-263. <https://doi.org/10.1177/2040206614536154>
- Mbwale I, Giliba RA, Kisula L, Kemboi D 2025. Unlocking nature's pharmacy: *Euphorbia hirta* (L.) as a potent defense against *Escherichia coli* and *Klebsiella pneumoniae* infections in Tanzania. *S Afr J Bot*, 177: 1-13. doi:10.1016/j.sajb.2024.11.036
- Mehta M, Agrawal AD, Bhansali S, Sharma R 2013. Hepatoprotective activity of Acorus calamus in paracetamol-induced liver damage in albino rats. *Asian Pacific Journal of Tropical Biomedicine*, 3(12): 965-969. [https://doi.org/10.1016/S2221-1691\(13\)60186-0](https://doi.org/10.1016/S2221-1691(13)60186-0)
- Meng XY, Zhang HX, Mezei M, Cui M 2011. Molecular docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design*, 7(2): 146-157.
- Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, 30(16): 2785-2791.
- Mukherjee PK, Biswas R, Sharma A, Banerjee S, Biswas S, Katiyar CK 2019. Validation of medicinal herbs for anti-diabetic activity. *Pharmaceutical Biology*, 53(9): 1316-1322.
- Negi AS, Kumar JK, Luqman S, Saikia D, Khanuja SPS, Gupta MM 2011. Acorus calamus: Chemistry, traditional and modern uses, and prospects. *Natural Product Communications*, 6(8): 1193-1202. <https://doi.org/10.1177/1934578X1100600826>
- Newman DJ, Cragg GM 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3): 770-803.
- Nijveldt RJ, van Nood E, van Hoor DE, Boelens PG, van Norren K, van Leeuwen PA 2001. Flavonoids: A review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition*, 74(4): 418-425.
- Pagadala NS, Syed K, Tuszyński J 2017. Software for molecular docking: A review. *Biophysical Reviews*, 9(2): 91-102. <https://doi.org/10.1007/s12551-016-0247-1>
- Panche AN, Diwan AD, Chandra SR 2016. Flavonoids: An overview. *Journal of Nutritional Science*, 5: e47.
- Prieto P, Pineda M, Aguilar M 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex. *Analytical Biochemistry*, 269(2): 337-341.
- Prior RL, Wu X, Schaich K 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10): 4290-4302.
- Rehman MU, Rather IA, Khan RA 2023. Natural antioxidants as emerging therapeutic interventions in hepatic disorders. *Antioxidants*, 12(4): 822. <https://doi.org/10.3390/antiox12040822>
- Rai N, Rai R, Raman V 2021. Asarone as a lead antiviral compound against SARS-CoV-2 main protease: An in silico approach. *Journal of Biomolecular Structure and Dynamics*, 39(18): 6935-6947. <https://doi.org/10.1080/07391102.2020.1835339>
- Rajput S, Baleti D, Vyas P, Bhavesh D 2014. Antiviral activities of plant-derived secondary metabolites: A review. *Asian Pacific Journal of Tropical Disease*, 4(1): 56-62. [https://doi.org/10.1016/S2222-1808\(14\)60319-7](https://doi.org/10.1016/S2222-1808(14)60319-7)
- Rawat S, Bouchard MJ 2015. The hepatitis B virus (HBV) HBx protein activates AKT to simultaneously regulate HBV replication and hepatocyte survival. *Journal of Virology*, 89(2): 999-1012.
- Ruch RJ, Cheng SJ, Klaunig JE 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10(6): 1003-1008.
- Saller R, Meier R, Brignoli R 2001. The use of silymarin in the treatment of liver diseases. *Drugs*, 61(14): 2035-2063.
- Schanderl SH 1970. *Methods in Food Analysis*. New York, USA: Academic Press.
- Shahidi F, Ambigaipalan P 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects. *Journal of Functional Foods*, 18(Part B): 820-897.
- Shaik AB, Shaik N, Panda SK, Abubakar SA, Abiramasundari P 2022. Molecular docking studies of bioactive fatty acid derivatives against viral non-structural proteins. *Journal of Molecular Modeling*, 28(3): 67. <https://doi.org/10.1007/s00894-022-05062-8>
- Sharma P, Jha AB, Dubey RS, Pessarakli M 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, 2012: 217037.
- Sindi ER, Polash MJ, Alves GB, et al. 2025. Assessment of *Annona muricata* phytochemicals as 17 β -HSD1 inhibitors through molecular docking, dynamics simulation, DFT, and ADMET analyses. *J Cell Mol Med*, 29(21): e70904. doi:10.1111/jcmm.70904.
- Singh A, Bhat TK, Sharma OP 2016. Clinical biochemistry of hepatotoxicity. *Journal of Clinical Toxicology*, 4(1): 1-19.
- Singh B, Kaur A, Arora S 2023. Pharmacological potential of saponins and glycosides in cardiovascular and immune-related disorders. *Biomedicine & Pharmacotherapy*, 163: 114765.
- Singleton VL, Orthofer R, Lamuela-Raventós RM 1999. Analysis of total phenols and other oxidation substrates

- and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299: 152-178.
- Sivasudhan E, Zhou J, Ma J, Wang Y, Liu S, Khan FI 2025. Hepatitis B virus X protein contributes to hepatocellular carcinoma via upregulation of KIAA1429 methyltransferase and mRNA m6A hypermethylation of HSPG2/Perlecan. *Mol Carcinog*, 64(1): 108-125. doi:10.1002/mc.23830.
- Sulaiman CT, Balachandran I, Narayanankutty A 2022. Advances in extraction and phytochemical characterization of medicinal plants using ethanol-based solvents. *South African Journal of Botany*, 146: 378-389. <https://doi.org/10.1016/j.sajb.2021.11.021>
- Taha MO, Daoud S 2026. Innovative integration of molecular docking and machine learning for drug discovery: From virtual screening to nanomolar inhibitors. *Chem Commun*. doi:10.1039/D5CC06025G
- Tanaka H, Dias JD, Jay B, Kita S, Sasaki M, Takeda H 2025. Structural basis of the hepatitis B virus X protein in complex with DDB1. *Proc Natl Acad Sci U S A*, 122(24): e2421325122. doi:10.1073/pnas.2421325122
- Tang H, Li Z, Yang G, Zhang Y, Wang Y 2023. HBx-mediated epigenetic dysregulation in hepatocellular carcinoma: Mechanisms and therapeutic implications. *Cell Communication and Signaling*, 21(1): 42. <https://doi.org/10.1186/s12964-023-01059-7>
- Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS, Bzowej NH, Wong JB 2018. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology*, 67(4): 1560-1599.
- Trease GE, Evans WC 2002. *Pharmacognosy*. 15th Edition. London, United Kingdom: Saunders Publishers.
- Trott O, Olson AJ 2010. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2): 455-461. <https://doi.org/10.1002/jcc.21334>
- Valko M, Leibfritz D, Moncol J, Cronin MTA, Mazur M, Telser J 2007. Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, 39(1): 44-84.
- Vijayakumar S, Sivaranjani M, Periakaruppan R, Sathiyarayanan A, Pushpalatha H 2021. Molecular docking analysis of fatty acid derivatives as potential inhibitors against dengue NS5 RNA-dependent RNA polymerase. *Journal of King Saud University – Science*, 33(6): 101554. <https://doi.org/10.1016/j.jksus.2021.101554>
- Wink M 2015. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*, 2(3): 251-286.
- World Health Organization 2023. *World Health Statistics 2023: Monitoring Health For The Sdgs, Sustainable Development Goals*.
- Zhang Y, Gan RY, Li S, Zhou Y, Li AN, Xu DP, Li HB 2022. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, 27(3): 789. <https://doi.org/10.3390/molecules27030789>

Paper received for publication in March, 2026
Paper accepted for publication in September, 2026