



OPEN LC/ESI-MS/MS phytochemical profiling and apoptotic effect of *Haloxylon scoparium* leaf extract on hepatocellular carcinoma

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Haloxylon scoparium, a plant native to Moroccan Sahara, was investigated for its potential anticancer activity against hepatocellular carcinoma (HCC). The study aimed to evaluate the effects of its methanolic extract on HCC and to conduct detailed chemical analysis using LC-ESI-MS/MS. *In vitro* cytotoxicity was assessed using HepG2 liver cancer cell line. *In vivo* experiments involved inducing HCC in mice with diethylnitrosamine (DEN). The study monitored inflammatory (TNF- α), apoptotic (BAX, Caspase-3, Caspase-8), and oncogenic markers (AFP, Bcl-2) through blood and liver tissue analysis. Liver histopathology was also performed to evaluate tissue-level changes. Mice survival rates were 83.33% in the DEN group and 91.67% in the DEN/*H. scoparium* group. Liver function markers (TBILR, ALP, AST) significantly decreased in the treatment group. TNF- α levels, elevated in DEN-only mice, were notably reduced after treatment. Oncogenic markers showed significant elevation in the DEN group but were decreased in the treatment group, whereas apoptotic markers were significantly elevated after treatment. Histopathology revealed more preserved liver architecture and scattered apoptotic foci in treated mice. Phytochemical profiling identified 27 compounds, including organic acids, phenolic acid derivatives, and flavonoids by LC/ESI-MS/MS. Molecular docking using AutoDock MGLTools 1.5.7 showed strong binding affinities of quercetin and isorhamnetin glycosides with cancer-related proteins (BCL-2, BAX, Caspases, AFP, TNF- α), supporting experimental results. 3D interaction models and box plots confirmed the stability and specificity of ligand–protein interactions. The study concludes that *H. scoparium* extract demonstrates promising multi-target anticancer potential and may serve as a valuable candidate for pharmaceutical development.

Keywords *Haloxylon scoparium*, Hepatocellular carcinoma, LC/ESI-MS/MS, Polyphenols, TNF- α , *In silico* study, Good health and well-being

Abbreviations

HCC	Hepatocellular carcinoma
LC/ESI-MS/MS	Liquid chromatography/electrospray ionization-mass spectrometry/ mass spectrometry
HepG2	Hepatocellular carcinoma
DEN	Diethylnitrosamine
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
TBILR	Total direct bilirubin

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TNF- α	Tumor necrosis factor alpha
LC-UV-MS/MS	Liquid chromatography-ultraviolet-mass spectrometry
BCL-2	B-cell lymphoma-2
AFP	Alpha-fetoprotein
i.p.	Intraperitoneally
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
cDNA	Complementary deoxyribonucleic acid
qPCR	Quantitative polymerase chain reaction
TBST	Tris-buffered saline with Tween [®] 20
PBS	Phosphate-buffered saline
SEM	Standard error of the mean
ANOVA	Analysis of variance
R _t	Retention time
IC ₅₀	Half maximal inhibitory concentration

Haloxylon scoparium (Pomel) Bge. [Synonyms: *H. articulatum* ssp. *scoparium* (Pomel) Batt., *Arthrophyllum scoparium* (Pomel) Iljin ex Maire & Weiller, *Hammada articulata* ssp. *scoparia* (Pomel) O.Bolòs & Vigo, and *Salsola articulata* Cav.] belongs to the family Chenopodiaceae of 120 genera and more than 1700 species¹.

Globally, they are distributed particularly in the salty desert and semi-desert areas in the form of herbs, subshrubs, and shrubs up to rarely small trees. The genus *Haloxylon* Bunge (Incl *Hammada*) includes about 25 species. It is distributed from the Western Mediterranean region to Arabia, Iran, Mongolia, Burma, and Southwest China^{2,3}. *H. scoparium* is a small, highly branched halophytic shrub distributed in sandy waste places of Northern Africa (Algeria, Libya, Morocco, Tunisia, Western Sahara, Egypt-Sinai; West tropical Africa, Mauritania) and Asia-Temperate: (Western Asia: Lebanon-Syria, Palestine)⁴. In traditional medicine, *H. salicornicum* is reported for diabetes⁵, as an antiseptic and anti-inflammatory⁶. In Oman, its stems are used as a mordant for dyeing wool in traditional weaving. In addition, *H. scoparium* is used to treat eye disorders¹. In Morocco, infusion and powder infusion of the aerial parts of *H. scoparium* are used for their antidiabetic effects⁷. This popular plant is widely used as a decoction, infusion, or cataplasm to treat hypertension, cutaneous neoplasms, dermatitis, diabetes, food poisoning, rheumatoid arthritis, osteoarthritis, scabies, injury healing, indigestion, stomachache, gastroenteritis, and cold⁸. Moreover, it is also used as an antidote to insects, scorpion stings, and snakebites. The leaves' decoction or infusion is used for the treatment of mouth diseases and toothache as a mouthwash^{1,9}.

Phytochemically, seven *Haloxylon* spp. have been investigated, leading to the isolation of several alkaloids belonging mainly to seven classes, i.e., aliphatic quaternary, pyridine, indole, isoquinoline, isoquinolone, β -carboline, and phenylethylamine alkaloids^{2,9}. Among 18 alkaloids, haloxynine was identified as a new piperidyl alkaloid from the aerial parts of *H. salicornicum*¹⁰. Also, sterols, flavonoid glycosides, and pyranones were reported in *Haloxylon* spp¹¹, which demonstrated cholinesterase and chymotrypsin inhibitory, antifungal, and nicotinic activities. It is worth mentioning that a review article reported the presence of 107 metabolites of different classes in *Haloxylon* spp. up to 2010^{12,13}. Scientists have analyzed *H. scoparium* for its phytochemical components along with biological effects. Multiple parts of the plant possess phenols together with flavonoids, followed by tannins, alkaloids, steroids, and saponins that result in its antioxidant potential and antibacterial effectiveness, as the extracted compounds from ethyl acetate demonstrated strong antibacterial properties against *Staphylococcus aureus*^{8,14}. In addition, an LC-UV-MS/MS analysis showed that *H. scoparium* contains both isorhamnetin and quercetin di- and triglycosides⁹.

Liver cancer is the seventh most common cancer in the world and one of the deadliest, with a 5-year survival rate ranging from 5 to 30%. According to the Global Burden of Disease Cancer Collaboration, one in every 38 men and one in every 111 women will develop liver cancer at some stage in their lives^{15,16}.

The current study aimed to investigate the *in vivo* anticancer activity of *H. scoparium* methanolic extract on hepatocellular carcinoma (HCC) by modulating the apoptotic pathway in the liver, along with the determination of its chemical profile using LC/ESI-MS/MS. Additionally, selected compounds identified in the extract were subjected to molecular docking to investigate their potential interactions with cancer- and apoptosis-related targets such as BCL-2, BAX, Caspase-3, Caspase-8, AFP, and TNF- α , to better understand their possible roles in the HCC modulation.

Materials and methods

Plant material

H. scoparium leaves were collected in January 2020 from the region of Aflou, located at the center of the Saharan Atlas (34°11' N; 02°10' E, 1372 m) in the Laghouat province of southern Algeria, under the relevant international guidelines and legislation, and with the necessary permissions obtained. The collection process adhered to standard practices for plant collection and preservation. The identification and authentication of the plant were carried out by Professor Mohamed KOUIDRI, Agricultural Department, Faculty of Science, Amar Telidji University, Laghouat, Algeria. A voucher specimen (No. H.s./2020) was deposited at the herbarium of the Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

Extract preparation

Dry powdered leaves of *H. scoparium* (1.2 kg) were soaked in 80% methanol (4 $\times$ 2 L) at room temperature. The combined extracts were filtered and evaporated under vacuum using a rotatory evaporator to afford a dry methanol extract of 265 g. Thereafter, it was defatted with pet. ether (60–80 °C) to afford 24.5 and 238.7 g dry samples from pet. ether-soluble portion and defatted extract, respectively.

***In vitro* anticancer activity of *H. scoparium* extract**

HepG2 cell line (ATCC: HB-8065) was provided by the Department of Cell Culture, Vacsera, Egypt. The cells were grown in a 1640 RPMI medium without PYR (Thermo Fisher Scientific, USA). The medium contained 10% FBS, 1% HEPES, and 1% antibiotic antimycotic mixture (LONZA). After attachment of the cells (7000 cells/well) in 96 tissue culture plate, different concentrations of the *H. scoparium* extract (1000, 500, 250, 125, 62.5, and 31.25 µg/ml) were experienced and the plates were incubated at 37 °C in 5% CO₂ for 24 h followed by staining using crystal violet method¹⁷.

***In vivo* studies**

Animals

The experiment was performed on male Swiss albino mice (*Mus musculus*); they were 6–8 weeks old and weighed 20 ± 15 g at the beginning of the experiment. They were provided by the animal house of Theodor Bilharz Research Institute (TBRI), Giza, Egypt. They were held in the same lab conditions of 20–25 °C, 50–60% relative humidity, and a 12-h light-dark period, with free access to normal food and water. All experiments were performed under the Institutional Ethical Committee.

Acute toxicity study

It was carried out to establish a safe *in vivo* dose. The extract was provided starting at 2 g/kg body weight (b.wt.) by oral gavage to mice (6 males, weight: 25–35 g, age: 6–8 weeks). For the first 4 h after dosing, the animals were monitored for toxic symptoms. Thereafter, the animals were kept for 72 h for observation of the death of mice, then maintained and observed daily for the next 13 days for any further toxicity^{18,19}.

DEN-induced HCC in Swiss albino mice

After acclimatization for one week, forty-eight mice were randomly allocated into 4 groups (12 mice/group)^{20–22} as follows:

- GpI: served as normal control and received 0.9% w/v NaCl intraperitoneally (*i.p.*).
- GpII: served as the HCC-induced group, DEN was administered *i.p.* at a dose of 24 mg/kg b.wt. in 0.9% NaCl (w/v) once/week, for 12 weeks.
- GpIII: was provided an oral administration of *H. scoparium* extract (200 mg/kg b.wt.) once per week for 12 weeks.
- GpIV: was subjected to DEN (24 mg/kg b.wt. in 0.9% NaCl) once/week for 8 weeks, then it was administered, orally, *H. scoparium* extract (200 mg/kg) once/week for 4 weeks along with DEN (24 mg/kg b.wt. in 0.9% NaCl, *i.p.*).

Assessed parameters for anticancer activity of *H. scoparium* in HCC-induced mice

Survival rate and body weight

The survival rate in each group was recorded and compared among all groups. In addition, the mice’s body weight was detected before scarifying, and its increase was compared among the animals of all groups.

Liver weight

At the end of the 12th week, the mice were sacrificed, and the liver was weighed to compare the relative liver weight among all groups.

Liver function test

The liver function test was performed using commercially available assay kits to measure serum aspartate aminotransferase (AST) and alanine transaminase (ALT) (Sclavo Diagnostics International), alkaline phosphatase (ALP) (N.S. BIO-TEC), and total direct bilirubin (TBILR) (Sclavo Diagnostics International).

Gene	Primer sequence	Reference
B-actin	Forward: GGGAATGGGTCAGAAGGACT	23
	Reverse: CTTCTCCATGTCGTCGCCAGT	
BCl-2	Forward: ATGCCTTTGTGGAACATATATGGC	24
	Reverse: GGTATGCACCCAGAGTGATGC	
BAX	Forward: CTACAGGGTTTCATCCAG	25
	Reverse: CCAGTTCATCTCCAATTCTG	
Caspase-8	Forward: TTCCGGATGAGGCAGACTTT	This study
	Reverse: CCTTGTTCCTCCTGTCGTCT	
Alpha-fetoprotein (AFP)	Forward: CTACATTTCGCTGCGTCCAA	This study
	Reverse: CAGCCAACACATCGCTAGTC	
Tumor necrosis factor-α (TNF-α)	Forward: ACCCTCACAACCTCACAACCA	26
	Reverse: GGCAGAGAGGAGGTTGACTT	

Table 1. Primers sequence for gene expression analysis using qPCR.

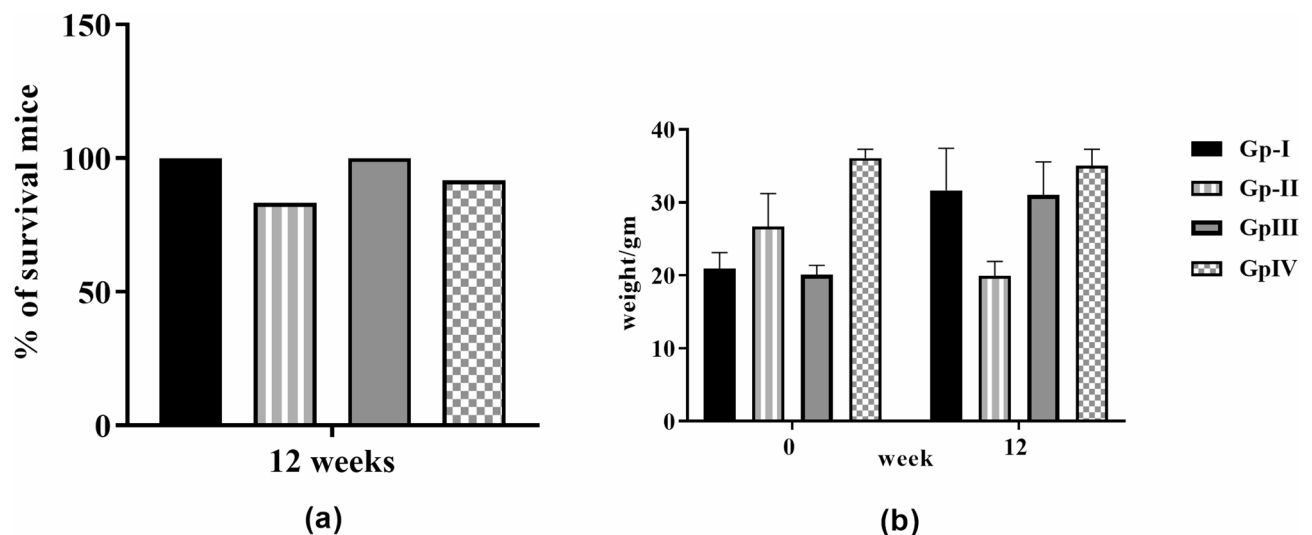


Fig. 1. (a) Percent of surviving mice at the end of the experiment (12 weeks). Results showed that *H. scoparium* post-treatment protected mice from death, and its dose was safe, as found in the *H. scoparium* group. (b) Effect of the *H. scoparium* extract on the b. w. of mice with HCC induced by DEN.

Week	Normal	DEN	<i>H. scoparium</i>	DEN/ <i>H. scoparium</i>
0	20.96 ± 0.62	26.69 ± 0.43	20.07 ± 1.3	36.07 ± 0.35
12	31.65 ± 1.66	19.9 ± 0.64	31.05 ± 1.36	35.06 ± 0.67

Table 2. Body weight of mice in each group.

Week	Normal	DEN	<i>H. scoparium</i>	DEN/ <i>H. scoparium</i>
12	1.42 ± 0.43	1.06 ± 0.31	1.18 ± 0.23	1.5 ± 0.37

Table 3. Liver weight of mice in each group.

Effect of H. scoparium on the apoptotic pathway in HCC-induced mice

Isolation of total RNA from serum and liver tissues was carried out using a commercially available kit (Biovision, Inc.). Quantitative PCR was performed using isolated RNA (1 µg), cDNA synthesis kit (Biovision, Inc.), and SYBR Green master mix (Thermo Fisher Scientific) to detect gene expression of tumor and apoptotic markers. A sequence of each primer was designed as in Table 1. The mean of each group was used to describe the expression ratio of each marker. The relative comparative quantitation approach was used to determine the gene expression levels ($RQ = 2^{-\Delta\Delta Ct}$).

Liver histopathology

The isolated livers were fixed in 10% buffered formalin for histopathological investigations. Liver pieces were processed routinely into paraffin blocks. A total of 4–5 µm thick sections were cut on positively charged glass slides. Sections were then stained with hematoxylin and eosin stain (H&E) for light microscopic histopathological examination regarding the hepatic architecture, inflammation, dysplasia, and malignancy. Masson trichrome stain was used for the assessment of tissue fibrosis. The liver histology from different groups was compared using an Axio microscope (Zeiss), and photos were taken using the attached digital Mrc5 camera (Zeiss).

Immunohistochemistry of caspase-3

Deparaffinizing and rehydrating the tissue pieces were done by immersing the slides in xylene with two changes for 10 min. each, followed by rehydration of tissue sections by immersing the slides in decreasing grades of ethanol. The slides were placed in a microwave-safe tray with 10 mM sodium citrate buffer (pH 6.0) and 0.05% Tween 20 to achieve antigen retrieval. The slides were then microwaved for 5 min., while being kept at a temperature just below boiling. The cooling process took 30 min. at room temperature. The slides were immersed in TBST 3 times, 3 min. each, to wash them (Tris Buffered Saline having 0.05% Tween 20). The slides were incubated in 3% hydrogen peroxide produced in methanol for 15 min., while they were kept in the dark to quench endogenous peroxidase. Following three TBST washes on each slide for 3 min. each, a 1:100 dilution of the Caspase-3 antibody (Rabbit anti-Caspase-3 antibody, Abcam Cat no. ab184787), in one volume of PBS, was

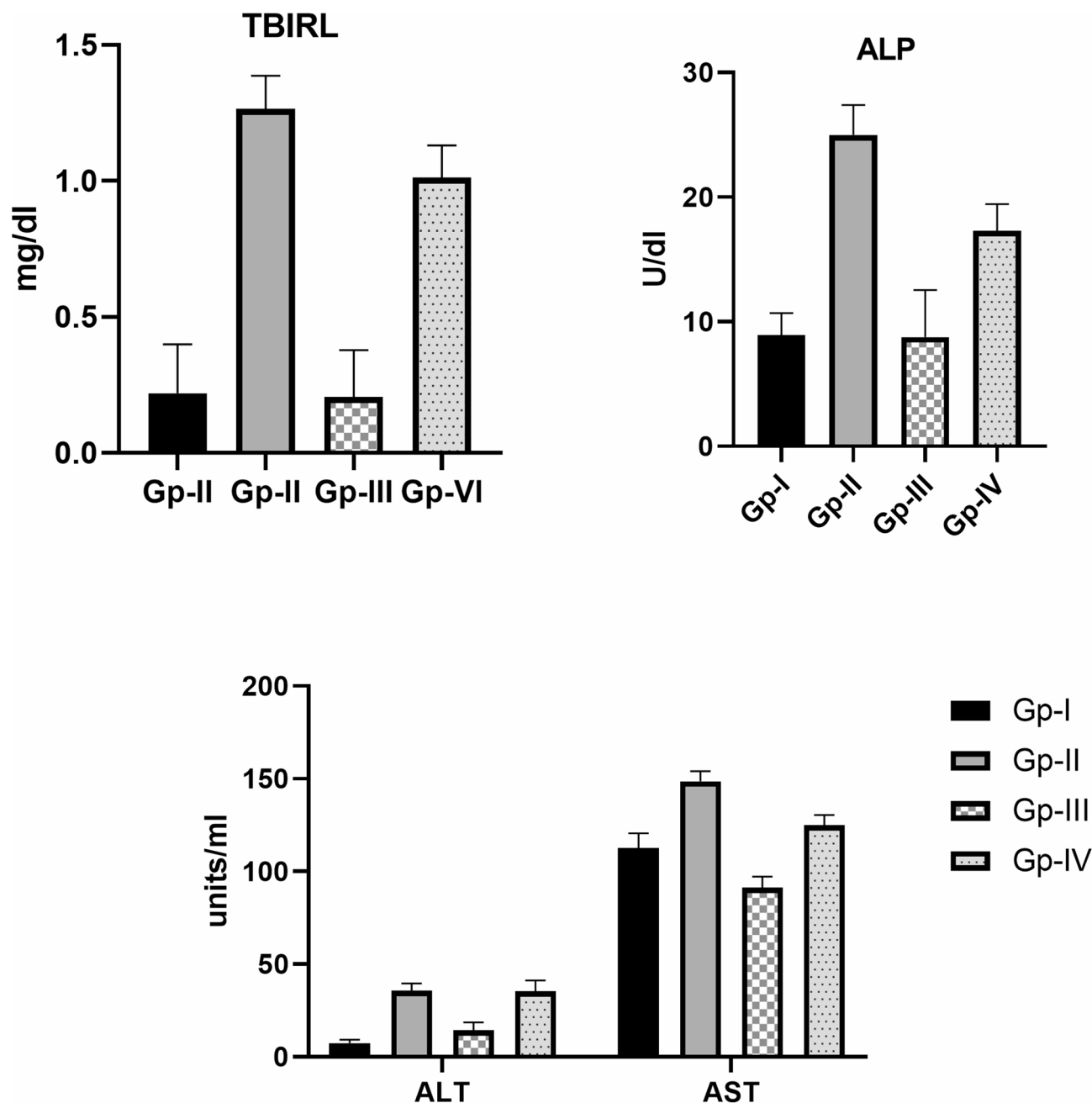


Fig. 2. Effect of the *H. scoparium* extract on the liver function in DEN-induced HCC in mice.

utilized. At 4 °C, in a humid environment, sections were incubated with diluted primary antibodies overnight. According to the manufacturer's recommendations, the sections were first washed 4 times, for 5 min. each, with TBST, followed by 30 min. of incubation with the secondary biotinylated antibody (Biotinylated Goat Anti-Rabbit IgG Abcam Cat no. ab64256) and another 30 min. with avidin-peroxidase complex. The expression level of Caspase-3 in tissue cells was judged according to the percentage of caspase-3 positive cells in each sample. Specifically, a percentage of $\leq 10\%$ was judged negative and $> 10\%$ was positive^{27,28}.

All sections were assessed and scored. The sections were examined by using a light microscope [Scope A1, Axio, Zeiss, Germany]. Photomicrographs were taken using a microscope camera [AxioCam, MRc5, Zeiss, Germany].

LC/ESI-MS/MS analysis

The chemical composition of *H. scoparium* leaf extract was performed using the LC/ESI-MS/MS technique in negative ion mode according to the reported procedures^{29,30}.

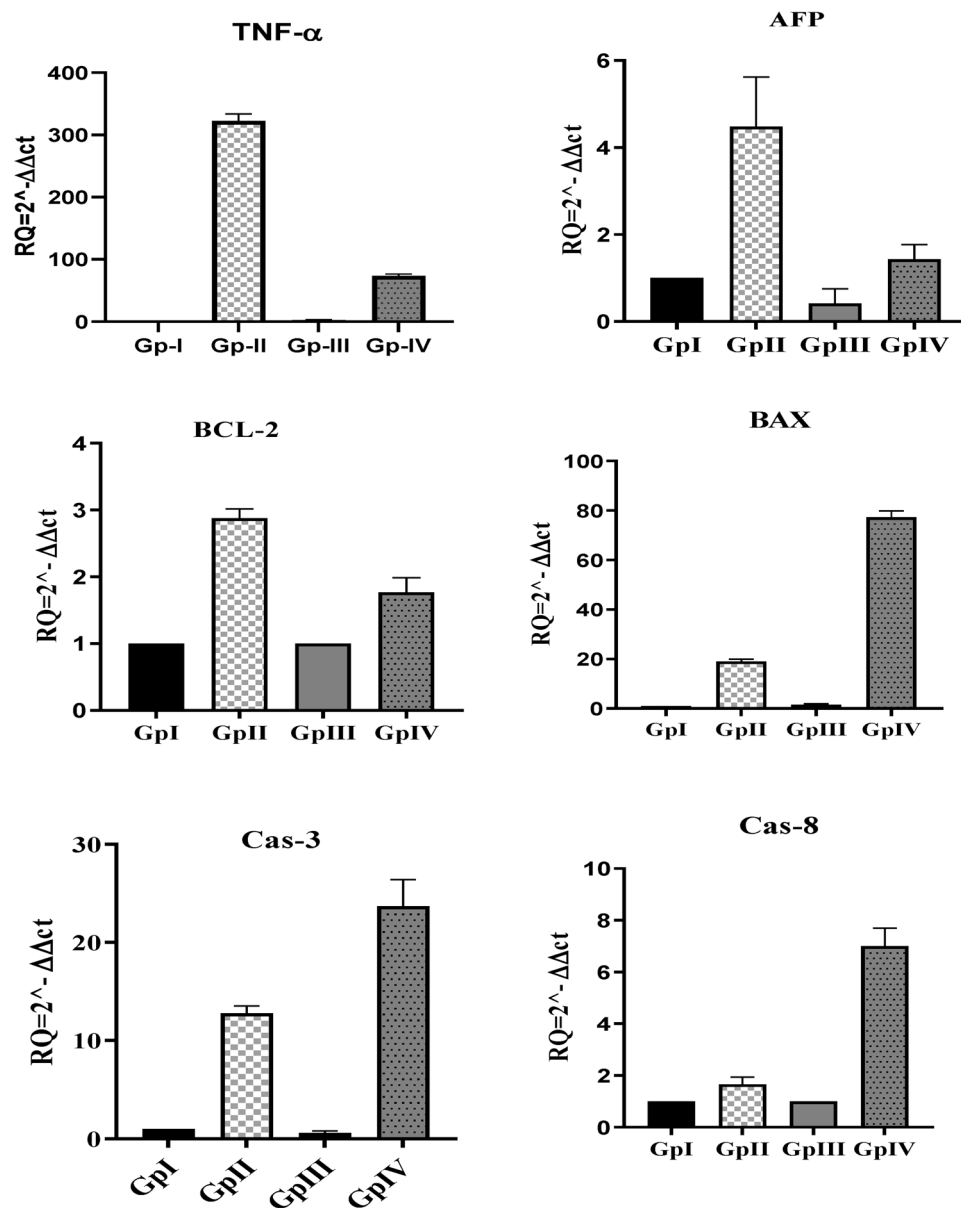


Fig. 3. Relative quantification of inflammatory marker (TNF- α), tumor markers (AFP and BCL-2), and apoptotic markers (BAX, Caspase-3, and Cas-8) in HCC-induced mice.

In silico studies

Several molecular targets play critical roles in the progression and regulation of hepatocellular carcinoma (HCC), making them valuable candidates for *in silico* docking studies. B-cell lymphoma 2 (Bcl-2) is an anti-apoptotic protein often overexpressed in HCC, contributing to tumor survival by inhibiting mitochondrial-mediated apoptosis³¹. In contrast, BAX, a pro-apoptotic member of the Bcl-2 family, promotes programmed cell death and is typically downregulated in liver cancer, thus aiding tumor evasion of apoptosis³². Caspase-8 functions as

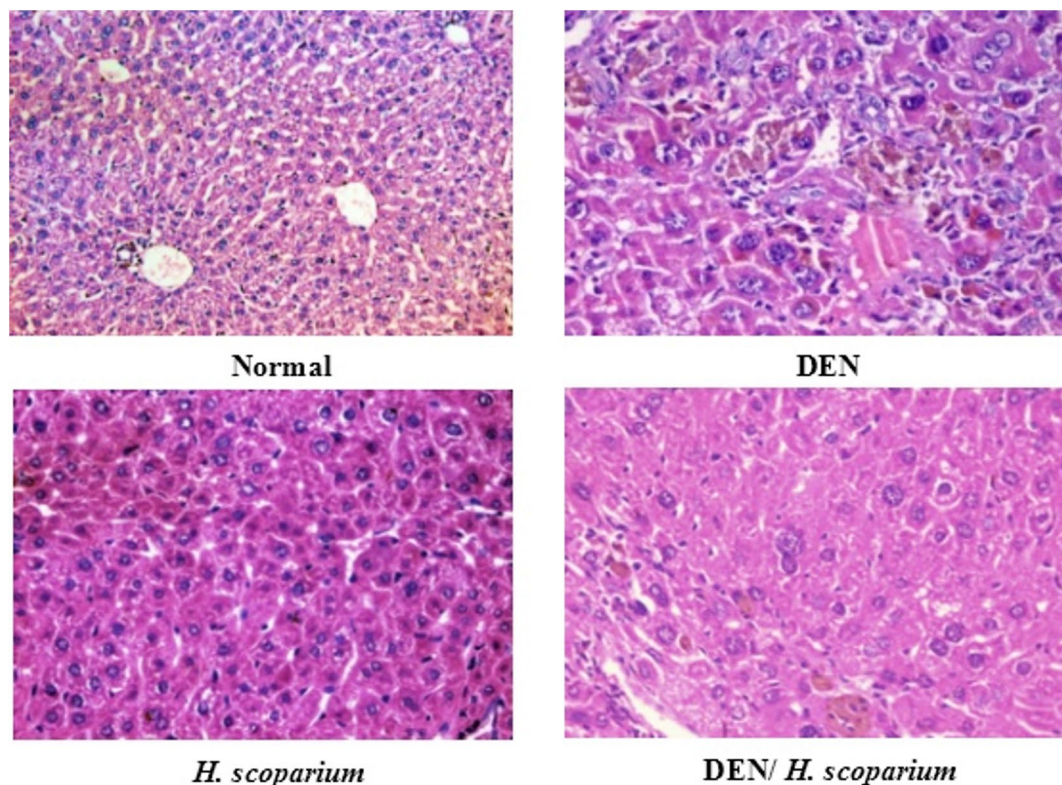


Fig. 4. Histopathology of the liver isolated from mice with DEN-induced HCC.

an initiator of the extrinsic apoptotic pathway and is frequently suppressed in HCC, facilitating immune escape and cell proliferation³³. Caspase-3, an executioner caspase, plays a crucial role in the terminal phase of apoptosis; its reduced activity in HCC is associated with enhanced tumor survival and chemoresistance^{34,35}. Alpha-fetoprotein (AFP) is a well-established HCC biomarker; beyond its diagnostic value, it is implicated in cell growth regulation, immune suppression, and oncogenic signaling³⁶. Additionally, tumor necrosis factor- α (TNF- α), a central pro-inflammatory cytokine, contributes to liver inflammation, fibrosis, and carcinogenesis and exhibits a complex dual role in both tumor suppression and promotion depending on the tumor microenvironment³⁶. These targets collectively represent key nodes in the apoptotic and inflammatory pathways dysregulated in HCC and thus are critical for computational docking and therapeutic exploration.

Ligand preparation

The study focused on bioactive compounds identified from *H. scoparium*, a plant known for its ethnomedicinal applications. All phytochemicals were selected, and control sorafenib was acquired from ChemDraw and the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in the form of 3D SDF format and subsequently converted to PDB format using Open Babel GUI software, ensuring compatibility with docking tools.

Target protein selection

Key apoptosis and cancer regulation proteins were selected as targets: Bcl-2 PDB ID:2W3L, BAX PDB ID:4S0O, Caspase-3 PDB ID:3GJQ, Caspase-8 PDB ID:4PRZ, AFP PDB ID:7YIM, and TNF- α PDB ID:2AZ5. These targets were chosen based on their established roles in cell survival and death signaling pathways and downloaded in the PDB file format from the RCSB Protein Data Bank (<https://www.rcsb.org/>).

Molecular docking

For docking, the proteins must be prepared by eliminating all water molecules, heteroatoms, and any Co-crystallized inhibitors. Additionally, hydrogen atoms and Kollman charges were added to the receptors using Autodock tools (ADT) (version 1.5.6). The docking process was performed using the AutoDock Vina program³⁷. The grid box was set to cover the entire active site of each protein. The best binding poses were selected based on binding energy (kcal/mol).

Visualization and analysis

Docked complexes were visualized using UCSF ChimeraX 1.9 and BIOVIA Discovery Studio Visualizer v 21, which facilitated both 3D and 2D interaction analysis. Box plots illustrating binding energy distributions were generated using OriginPro 2025.

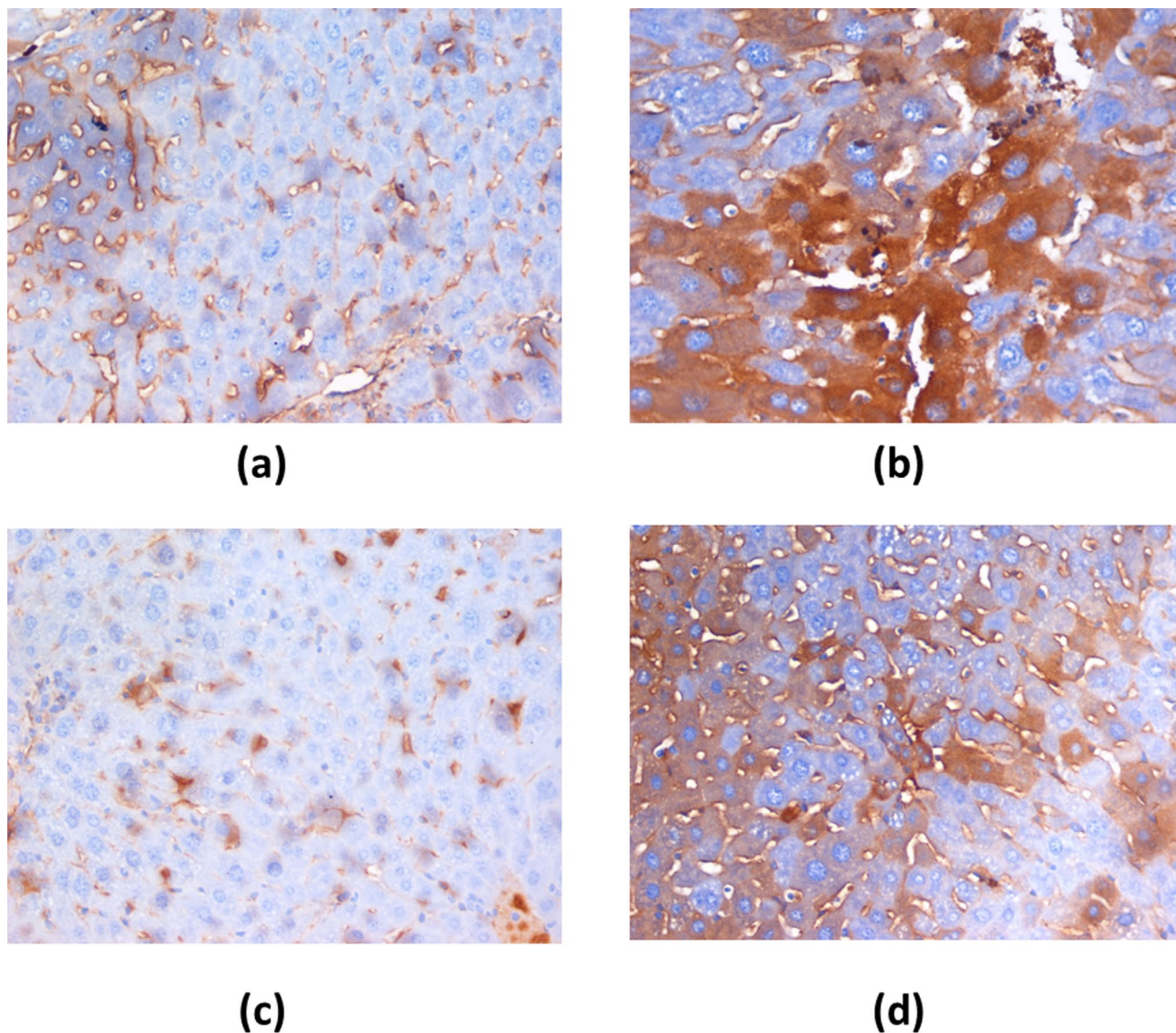


Fig. 5. Immunohistochemistry of Caspase-3 protein detection (X400). (a) Section in the liver of Normal mice showing predominantly sinusoidal expression of Caspase-3 with moderate intensity. (b) Section in the liver of DEN-treated mice showing remarkable dense expression of Caspase-3 within hepatocytes (brownish discoloration). (c) Section in the liver of HS-treated mice showing focal mild expression of Caspase-3 within hepatocytes and sinusoids. (d): Section in the liver of HS + DEN-treated mice showing increased dense expression of Caspase-3 within hepatocytes.

	Normal GpI	DEN GpII	<i>H. scaparium</i> GpIII	DEN/ <i>H. scaparium</i> GpIV
Percentage (M $\pm$ SD)	1.05 $\pm$ 0.33	22.87 $\pm$ 5.16	3.04 $\pm$ 0.24	15.36 $\pm$ 4.55
Density score	++	+++	++	+++

Table 4. Caspase-3 Immunohistological expression in each group.

Statistical analysis

Data in the treatment groups were presented as mean $\pm$ SEM, and statistical analysis was performed using GraphPad Prism 8. One-way or Two-way ANOVA was followed by post hoc Tukey's multiple comparison tests. It was determined that the $P < 0.05$ was statistically significant.

Tested extract	TPC (mg GAE/g dry extract)	TFC (mg RE/g dry extract)
<i>H. scoparium</i>	157.67 ± 4.84	65.57 ± 1.89

Table 5. Total phenolic (TPC) and total flavonoid (TFC) contents of the *H. scoparium* leaf extract. Data are presented as mean ± S.D., *n* = 3. GAE: gallic acid equivalent; RE: rutin equivalent.

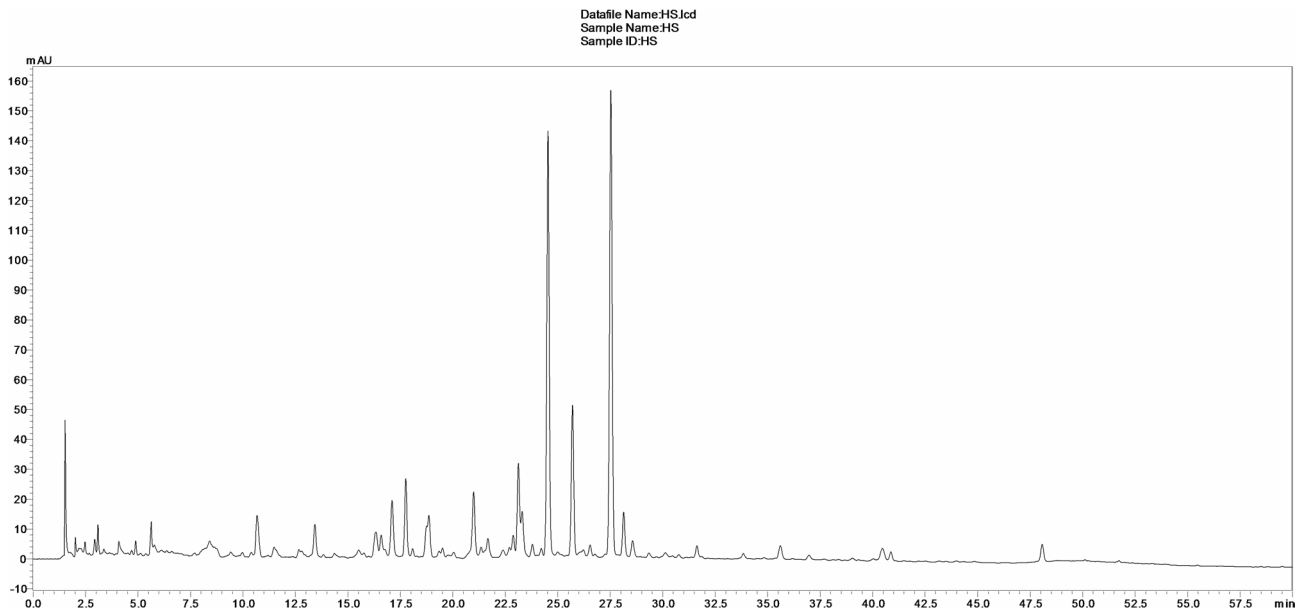


Fig. 6. TIC chromatogram from LC/ESI-MS/MS analysis of *H. scoparium* leaf extract.

Results

In vitro cytotoxicity of *H. scoparium* against the HepG2 cell line

The calculated IC₅₀ was 282.4 ± 35.88 µg/ml. A statistical significance was detected among different concentrations (1000, 500, 250, 125, 62.5, and 31.25 µg/ml), using One-way ANOVA (*p* < 0.0001).

Acute toxicity study

There was no mortality or apparent toxicity from a dosage of 2 g/kg b.wt., such as diarrhea, urination, edema, or skin color change. Also, after 14 days of observation, there was no mortality in these mice. On a dosage of 200 mg/kg b.wt. orally, the extract was considered to be non-toxic based on these observations and the literature.

Assessed parameters for anticancer activity of *H. scoparium* in HCC-induced mice

Survival rate and body weight

The survival of mice in each group was monitored throughout the experiment. Results showed that no death was found in both normal and *H. scoparium* groups. Nevertheless, 83.33 and 91.67% of mice survived in DEN and DEN/*H. scoparium* groups, respectively (Fig. 1a). In Table 2; Fig. 1b, the variation in the b.wt. was recorded among all groups at the beginning of the experiment (0 weeks) and before sacrificing (12 weeks). There was a significant reduction in the b.wt. in the DEN group compared to the normal group. Furthermore, the b.wt. increased significantly in all groups, and the *H. scoparium* extract did not prevent this increase, showing a pattern similar to the normal group. Besides, post-treatment in DEN/*H. scoparium* group showed that the extract significantly prevented the loss of the b.wt. compared to the normal and DEN groups (*p* < 0.0001).

Liver weight

Analysis using One-way ANOVA followed by hoc Tukey’s multiple comparison tests showed that there was a statistical significance between normal and DEN groups (*p* = 0.0034) and between DEN and DEN/*H. scoparium* groups (*p* = 0.045). However, there was no significant difference between *H. scoparium* and DEN/*H. scoparium* or normal and *H. scoparium* (Table 3).

Liver function test

There was a significant increase in the level of AST, ALT, and ALP in the DEN group compared to the normal group, and no significant difference in TBILR was recorded. In the *H. scoparium* group, a significant difference was observed in the level of AST and ALT compared to both normal and DEN groups. Moreover, findings referred to a significant decrease in TBILR, ALP, and AST levels of DEN/*H. scoparium* group compared to the DEN group (Fig. 2).

R _t	[M-H] ⁻	MS/MS	Tentative identification	Ref.
1.45	191	173, 129, 111, 87, 57	Citric acid	38
1.57	133	115, 89, 71	Malic acid	39
3.68	331	313, 271, 169, 125	Galloylglucose	40
4.39	331	313, 271, 169, 125	Galloylglucose	40
5.06	315	153, 109, 81, 42	Gentisic acid glucoside	41
5.12	329	153, 109, 81, 42	Gentisic acid glucuronide	42
5.72	463	331, 169, 151, 125	Galloyl-pentosyl glucose	43
5.84	285	153, 109, 108	Gentisic acid pentoside	44
6.44	301	169, 125	Galloylpentose	45
6.56	261	181, 179, 137, 109	Dihydrocaffeic acid sulphate	46
7.70	343	191, 169, 125, 111, 107	Galloylquinic acid	47
8.18	285	153, 109, 108	Gentisic acid pentoside	44
10.67	417	285, 241, 153, 109, 108	Gentisic acid dipentoside	48,49
13.11	153	135, 123, 109, 108	Gentisic acid	50
13.42	369	193, 178, 149, 134, 119	Ferulic acid 4-O-glucuronide	51
16.33	785	639, 623, 459, 315, 300, 271, 151	Isorhamnetin 3-O-rutinoside-7-glucoside	52
17.68	917	873, 669, 505, 315, 314, 299	Isorhamnetin-O-hydroxyferuloylhexoside-O-malonylhexoside (isomer 1)	52
18.85	917	873, 669, 505, 315, 314, 299	Isorhamnetin-O-hydroxyferuloylhexoside-O-malonylhexoside (isomer 2)	52
20.98	741	609, 595, 301, 300, 255, 179, 151	Quercetin-O-pentoside-O-deoxyhexoside-hexoside	52,53
23.12	725	563, 315, 314, 299	Isorhamnetin malonyldiglucoside	54
24.53	741	609, 595, 301, 300, 255, 179, 151	Quercetin-O-pentoside-O-deoxyhexoside-hexoside	52,53
25.69	755	623, 461, 315, 314, 300, 299, 255, 179, 151	Isorhamnetin-O-deoxyhexosyl-pentosyl-hexoside	55,56
27.52	623	477, 461, 315, 314, 300, 299, 255, 179	Isorhamnetin-O-rutinoside (isomer 1)	57
28.13	623	477, 461, 315, 314, 300, 299, 255, 179	Isorhamnetin-O-rutinoside (isomer 2)	57
28.50	477	315, 314, 300, 299, 255, 179, 151	Isorhamnetin 3-O-glucoside	58
30.71	515	353, 335, 191, 179, 173, 151, 135	Dicaffeoylquinic acid	29,59
34.81	431	311, 269, 225	Apigenin 7-O-glucoside	60

Table 6. Chemical composition of the *H. scoparium* leaf extract by LC/ESI-MS/MS analysis.

Effect of *H. scoparium* on the apoptotic pathway in HCC-induced mice

The relative expression of serum TNF- α was significantly increased in the DEN group (322.44 ± 11.2) compared to the normal group (0.07 ± 0.02). In the *H. scoparium* group, the relative expression was 2.48 ± 0.6 . However, it was significantly decreased in the DEN/*H. scoparium* group (73.98 ± 2.7) compared to the DEN group. The relative expression level of tumor markers Bcl-2 and AFP was significantly increased in the DEN group compared to the other groups. In addition, apoptotic markers BAX, Caspase-3, and Cas-8 were significantly increased in the DEN/*H. scoparium*-treated group compared to the normal and DEN groups (Fig. 3).

Effect of *H. scoparium* extract on the treatment of HCC supported by histopathology and immunohistochemistry

Histopathology is depicted in a photograph (Fig. 4). Hepatocytes (with granular cytoplasm that took up the acidophilic stain) and nuclei (centrally located) were seen in the histology of both normal and *H. scoparium* groups. With H&E staining, the central vein and bile ducts can be seen clearly. Together with areas of necrosis, cholestasis, bile duct proliferation, and lymphatic dilatation, a distorted architecture, focal HCC, and dysplasia are seen in the histology of the DEN group. However, the hepatic architecture is more preserved with minor hepatocytic changes in the post-treated DEN/*H. scoparium* group, but with scattered foci of hepatocytic apoptosis. On the other hand, the *H. scoparium*-treated group showed more or less normal hepatic architecture with no histopathological changes. Results of IHC showed a mild expression of Caspase-3 in both normal and *H. scoparium* groups. However, an increased expression of Caspase-3 was detected in both DEN and DEN/*H. scoparium* groups as shown in Fig. 5; Table 4.

The normal group shows a preserved hepatic lobular architecture with the absence of degeneration, inflammation, or fibrosis. DEN group shows HCC with focal acinar formation and bizarre-shaped hyperchromatic nuclei with focal cholestasis and distorted lobular pattern. *H. scoparium* group shows preserved hepatic lobular architecture with focal cholestasis and mild hepatocytic hydropic changes. DEN/*H. scoparium* group shows focal dysplasia, few apoptotic figures, and cholestasis.

Total phenolic (TPC) and total flavonoid (TFC) contents

As illustrated in the Table 5, the *H. scoparium* leaf extract exhibited substantial total phenolic (TPC) and total flavonoid (TFC) contents when examined using the Folin-Ciocalteu's and aluminum chloride assays, respectively. The TPC was determined to be 157.67 ± 4.84 mg GAE/g dry extract. Also, the TFC was determined to be 65.57 ± 1.89 mg RE/g dry extract.

Compound	BCL-2	BAX	Caspase-3	Caspase-8	AFP	TNF- α
Citric acid	-5.1	-5.3	-4.6	-4.7	-5.9	-5.2
Malic acid	-4.4	-4.6	-4.8	-4.9	-5.1	-4.3
Galloylglucose	-7.0	-7.1	-6.5	-7.1	-7.1	-6.7
Gentisic acid glucoside	-6.6	-6.8	-6.4	-6.7	-7.6	-7.8
Gentisic acid glucuronide	-6.7	-8.0	-6.5	-6.4	-7.9	-7.2
Galloyl-pentosylglucose	-8.5	-7.3	-7.9	-8.3	-8.4	-8.7
Gentisic acid pentoside	-6.5	-7.0	-6.0	-6.2	-7.2	-7.6
Galloylpentose	-7.1	-7.5	-7.2	-7.6	-7.7	-7.4
Dihydrocaffeic acid sulphate	-6.0	-6.0	-5.6	-6.2	-7.0	-6.6
Galloylquinic acid	-7.2	-7.0	-6.2	-6.6	-7.3	-6.8
Gentisic acid dipentoside	-8.9	-7.9	-8.0	-7.5	-8.7	-8.9
Gentisic acid	-5.7	-5.7	-4.7	-5.1	-6.9	-5.6
Ferulic acid 4-O-glucuronide	-5.6	-6.4	-5.0	-5.8	-6.0	-6.6
Isorhamnetin 3-O-rutinoside-7-glucoside	-10.4	-8.1	-9.0	-8.5	-10.1	-9.6
Isorhamnetin malonyl- diglucoside	-10.3	-8.4	-9.3	-9.1	-9.4	-10.5
Quercetin-O-pentoside-O-deoxyhexoside-hexoside	-10.5	-10.2	-9.4	-10.5	-9.9	-10.4
Isorhamnetin-O-deoxyhexosyl-pentosyl-hexoside	-10.2	-8.4	-9.7	-9.6	-9.9	-10.6
Isorhamnetin 3-O-rutinoside	-8.7	-7.7	-7.7	-9.2	-8.6	-8.9
Isorhamnetin 3-O-glucoside	-9.0	-9.0	-8.3	-9.0	-8.4	-9.2
Dicaffeoyl quinic acid	-9.0	-8.1	-7.1	-8.4	-7.8	-8.4
Apigenin 7-O-glucoside	-7.6	-7.9	-7.1	-7.3	-8.3	-8.2
Sorafenib (Control)	-8.1	-7.8	-7.8	-8.0	-9.8	-9.5

Table 7. Docking scores (binding energies in kcal/mol) of the compounds with apoptosis and cancer-related targets.

Chemical profiling of the *H. scoparium* leaf extract

The LC/ESI-MS/MS examination of *H. scoparium* leaf extract furnished 27 compounds comprising organic acids, phenolic acid derivatives, and flavonoids (Fig. 6; Table 6). Interestingly, two organic acids were annotated in the tested extract. They furnished $[M-H]^-$ anions at m/z 191, and 133, and daughter ions at 111 $[M-H-80]^-$, and 115 $[M-H-18(H_2O)]^-$; they were assigned as citric, and malic acids, respectively. A characteristic set of gallic acid derivatives was observed in the tested extract. They disclosed their own molecular ions ($[M-H]^-$) at m/z 331, 463, 301, and 343, and daughter ions at 169 $[M-H-162(Glu)]^-$, 331 $[M-H-132(pentose)]^-$, 169 $[M-H-132(pentose)]^-$, and 191 $[M-H-152(galloyl\ moiety)]^-$. They were assigned as galloylglucose, galloyl-pentosylglucose, galloylpentose, and galloylquinic acid, respectively. As well, another set of gentisic acid derivatives was also detected in the tested extract. They exhibited molecular ions $[M-H]^-$ at m/z 315, 329, 285, and 417, with identical daughter ions at 153 $[M-H-162(Glu)]^-$, 153 $[M-H-176(Glu)]^-$, 153 $[M-H-132(pentose)]^-$, and 153 $[M-H-264(2\ pentose)]^-$. They were identified as the glucoside, glucuronide, pentoside, and dipentoside of gentisic acid, respectively. Another peak ($R_t = 13.11$) demonstrated a molecular ion at m/z 153 $[M-H]^-$ and a decarboxy daughter ion at 109 $[M-H-44(CO_2)]^-$ that was interpreted as gentisic acid. Three signals demonstrated their molecular ions ($[M-H]^-$) at m/z 261, 369, and 515 and daughter ions at 181 $[M-H-80(sulfate\ moiety)]^-$, 193 $[M-H-176]^-$, and 191 $[M-H-324(2\ caffeoyl\ moiety)]^-$ that were tentatively identified as dihydrocaffeic acid sulphate, ferulic acid 4-O-glucuronide, and dicaffeoylquinic acid, respectively. Moreover, a set of isorhamnetin glycosides was annotated in the tested extract. They furnished deprotonated ions ($[M-H]^-$) at m/z 917, 725, 755, 623, and 477, with identical aglycone ions at 315 $[M-H-602]^-$, 315 $[M-H-410]^-$, 315 $[M-H-440]^-$, 315 $[M-H-308(rutinoside\ moiety)]^-$, and 315 $[M-H-162(Glu)]^-$. In addition their spectra disclosed also two characteristic daughter ions at 314 and 299 amu that were interpretable for the common online oxidation of the previous aglycone fragment and demethylation, respectively. Accordingly, they were assigned as isorhamnetin-O-hydroxyferuloylhexoside-O-malonylhexoside, malonyldiglucoside, triglycoside, rutinoside, and glucoside of isorhamnetin, respectively. The other four flavonoid derivatives were picked up from the TIC of the tested extract ($R_t = 16.33, 20.98, 24.53, 34.81\ min.$). They disclosed $[M-H]^-$ ions at m/z 785, 741, 741 and 431 with daughter ions at 315 $[M-H-470]^-$, 301 $[M-H-440]^-$, 301 $[M-H-440]^-$, and 269 $[M-H-162]^-$, assignable for isorhamnetin glycoside, two quercetin triglycosides, and an apigenin glucoside.

Docking scores and best binding interactions

The molecular docking results provide strong evidence that *H. scoparium*-derived flavonoid glycosides, especially quercetin and isorhamnetin glycosides (Table 7), exhibit high binding affinities toward multiple apoptosis and cancer-related targets. Quercetin triglycoside showed the most robust interaction profile, particularly with BCL-

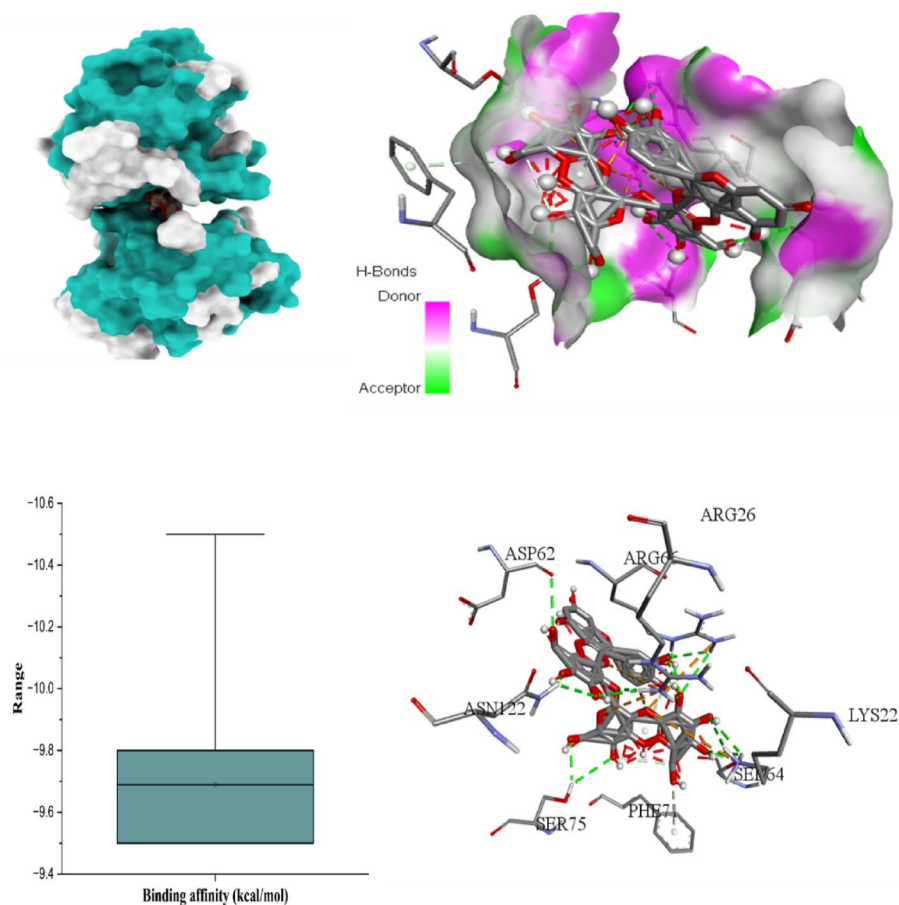


Fig. 7. 3D binding interaction and Box plot representing the docking score (binding affinity) in kcal/mol of Quercetin triglycoside with the BCL-2 protein.

2 (-10.5 kcal/mol), BAX (-10.2 kcal/mol), Caspase-8 (-10.5 kcal/mol), and TNF- α (-10.4 kcal/mol), suggesting its potential as a broad-spectrum inhibitor of both pro- and anti-apoptotic proteins. Similarly, isorhamnetin triglycoside showed exceptional affinity for TNF- α (-10.6 kcal/mol), making it a promising candidate for modulating inflammatory signaling.

The six figures illustrate the molecular docking interactions of potent *H. scoparium* compounds with key cancer- and apoptosis-related targets, highlighting both binding affinity and specific residue interactions. Figure 7 shows quercetin triglycoside bound to BCL-2, where the ligand forms hydrogen bonds with residues such as LYS22 (2.73 Å), ARG26 (3.35 Å), SER64 (3.11 Å), ARG66 (1.97 Å), ASP62 (2.93 Å), SER75 (2.29 Å), and ASN122 (2.10 Å). Figure 8 depicts its binding to BAX, engaging GLN28 (2.54 Å), GLN32 (3.08 Å), GLU41 (2.73 Å), ALA42 (3.30 Å), and LEU47 (4.02), suggesting the potential to influence BAX-mediated apoptotic signaling. Figure 9 presents isorhamnetin triglycoside interacting with Caspase-3, anchored by hydrogen bonds and π - π stacking with residues such as GLN217 (2.18 Å), TRP214 (2.08 Å), ASN208 (2.13 Å), ARG207 (2.78 Å), and PHE250 (4.74 Å), possibly enhancing caspase activation. In Fig. 10, quercetin triglycoside exhibits strong interaction with Caspase-8, forming key bonds with residues like SER256 (2.46 Å), ARG258 (2.62 Å), HIS317 (2.38 Å), GLY 318 (2.94 Å), SER411 (2.80 Å), and ARG413 (2.40 Å), indicating a role in extrinsic apoptotic pathway regulation. Figure 11 shows that AFP interacts with isorhamnetin 3-O-rutinoside-7-glucoside and forms two H-bonds, LEU365 (2.35 Å) and VAL367 (2.45 Å). Figure 12 highlights isorhamnetin triglycoside in complex with TNF- α , anchored by interactions with GLN125 (2.68 Å), suggesting inhibition of inflammatory signaling. The box plots accompanying each figure visually reinforce the binding affinity trends, with lower binding energies indicating stronger and more stable interactions. These figures collectively support the therapeutic potential of glycosylated flavonoids as multi-target agents acting on apoptotic and inflammatory pathways.

Discussion

It is worth mentioning to refer that out of the 27 metabolites characterized in this report by LC/MS, 9 metabolites only were identified before from *H. scoparium*, while the remaining 18 ones were for the first time from the

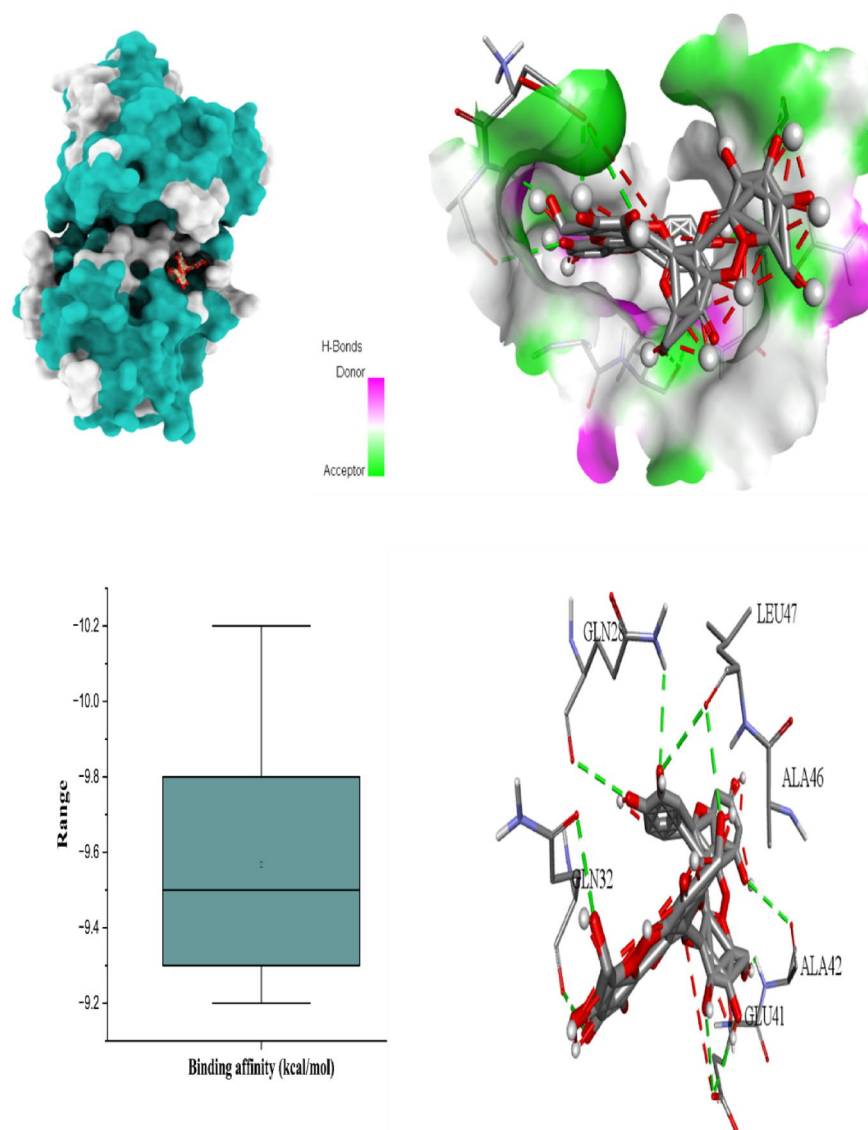


Fig. 8. 3D binding interaction and Box plot of Quercetin triglycoside with the BAX protein.

Haloxylon genus. The metabolites previously reported are gentisic acid⁶¹, isorhamnetin triglycoside (rhamnosyl-pentosylglucoside)⁵⁵, apigenin 7-*O*-glucoside⁶², isorhamnetin-*O*-hexoside-*O*-deoxyhexosyl-hexoside⁵², isorhamnetin-*O*-hydroxyferuloylhexaside-*O*-malonylhexaside⁵², quercetin-*O*-pentoside-*O*-deoxyhexoside-hexoside⁵², isorhamnetin-*O*-deoxyhexosyl-pentosyl-hexoside⁵², isorhamnetin 3-*O*-rutinoside⁵², and isorhamnetin 3-*O*-glucoside⁵².

While there are few direct reports on the anticancer activity of *H. scoparium*, indirect evidence from other close relatives of the *Haloxylon* genus and the broader Amaranthaceae/Chenopodiaceae family suggests its potential as a therapeutic compound. Methanol extract of the *H. salicornicum* was reported to induce apoptosis in cancer cells with DNA damage in the morphology of the cells. This plant possesses similar phytochemical profiles to *H. scoparium*⁶³. Ethnopharmacological history also documents the traditional use of *H. scoparium* in the treatment of inflammatory disease^{9,64}, suggesting probable immunomodulatory activity that could be implicated in antitumor action. To achieve our goal, firstly we detected its cytotoxic effect on the HepG2 cell line. The results showed that the calculated IC_{50} was found to be $282.4 \pm 35.88 \mu\text{g/ml}$, which did not meet the NCI's stringent cut off for the *in vitro* activity ($IC_{50} \leq 30 \mu\text{g/mL}$)^{63,65}. The fact that it exhibited significant antitumor activity *in vivo* suggests that its mechanism of action may be beyond direct cytotoxicity. This disparity highlights a well-reported limitation of *in vitro* models, which fail to reflect the complex tumor microenvironment, metabolic activation, and systemic immune response that are all components of a compound's overall therapeutic

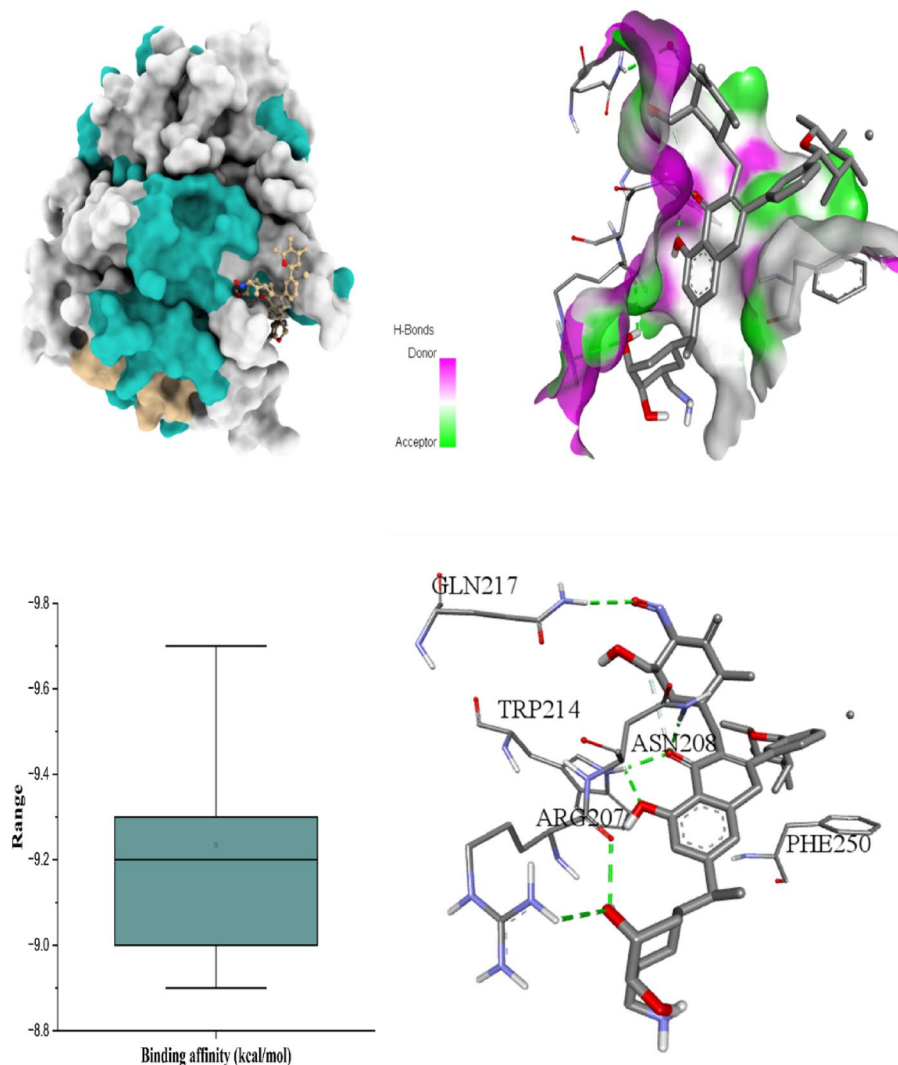


Fig. 9. 3D binding interaction and Box plot of Isorhamnetin triglycoside with Caspase-3.

potential *in vivo*⁶⁶. The majority of natural products possess multi-target mechanisms of anticancer activity, e.g. immunomodulation, anti-angiogenesis, or inhibition of tumor-stroma interactions—that are impossible to capture in monolayer cell cultures^{67,68}. Thus, while *in vitro* screening is giving useful initial information, *in vivo* efficacy of the extract supports its promise for consideration in future mechanistic and preclinical studies. Based on these findings and the literature, it is determined that the extract is non-toxic *in vivo* at a dose of 200 mg/kg b.wt., orally. Herein, the HCC model was established using DEN, which is considered a liver toxin that causes liver cancer⁶⁹. Development of HCC followed by treatment with *H. scoparium* extract showed a substantial reduction in body weight loss compared to the DEN group. Correspondingly, at the end of the experiment, 83.33% and 91.67% of mice survived in DEN and DEN/*H. scoparium* groups, and no death was detected in the normal or *H. scoparium* group. After sacrificing, the liver weight in the DEN group was less than the post-treated group ($p = 0.045$). DEN exposure resulted in the release of stress-specific enzymes in the blood, as shown by substantial increases in liver functional markers such as ALT, AST, bilirubin, and ALP^{21,70}. In this study, the DEN group revealed an increase in the AST, ALT, ALP, and TBILR levels compared to other groups. This is due to severe hepatocyte necrosis, bile duct proliferation, and cholestasis, leading to both hepatocellular damage and impaired bile excretion. However, the TBILR, ALP, and AST were decreased in DEN/*H. scoparium* group relative to the DEN group, indicating liver recovery. The release of cytochrome-C or other caspase-activating factors from the mitochondrial intermembrane space into the cytoplasm is a cornerstone of the intrinsic apoptosis pathway. This release is dependent upon the mitochondrial permeability transfer pore. The apoptosome is then produced in the cytoplasm that activates caspase-9, and then activates the effector caspase cascade^{71,72}. The level of Bcl-2 as a carcinogenic marker was significantly increased in the DEN group compared to other groups. In addition, apoptotic markers BAX, Caspase-3, and Cas-8 were significantly increased in DEN/*H. scoparium*-treated group

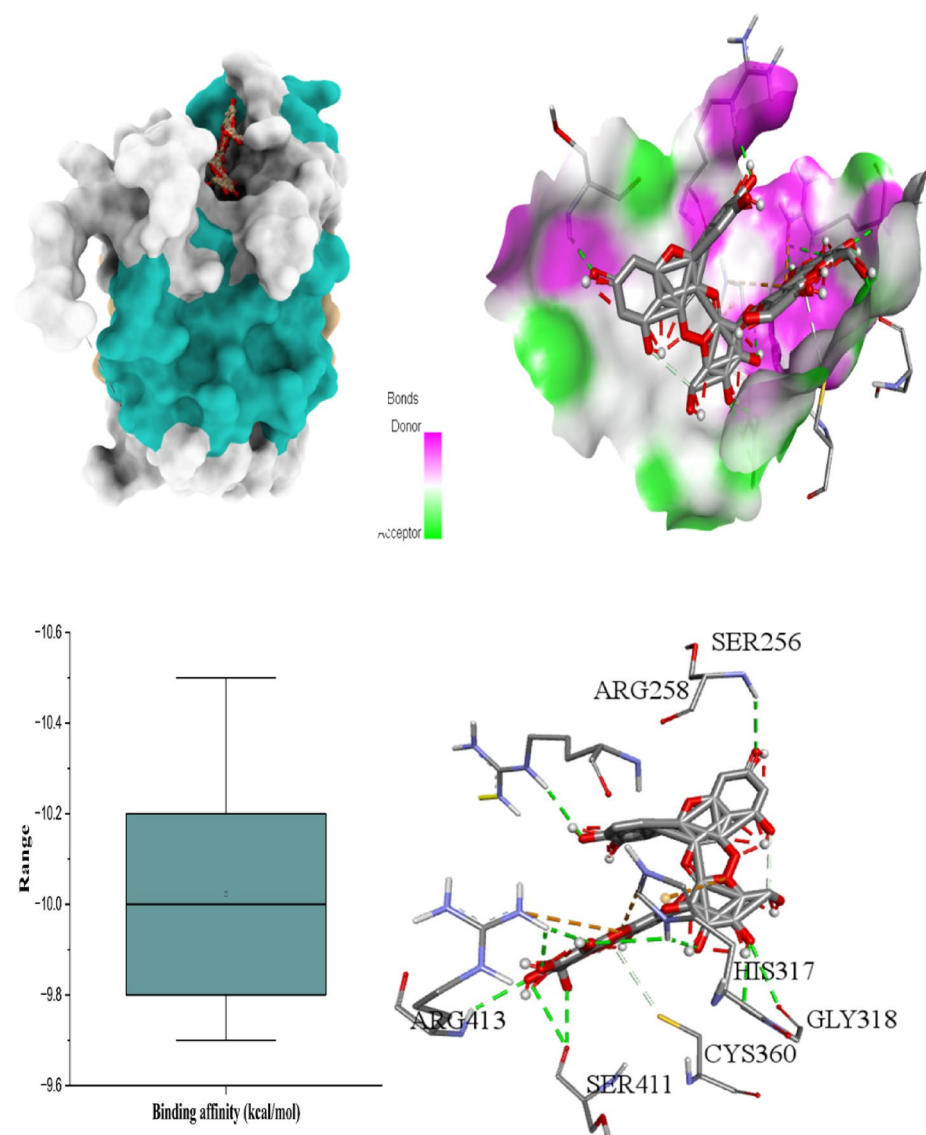


Fig. 10. 3D binding interaction and Box plot of Quercetin triglycoside with Caspase-8.

compared to the normal and DEN groups. AFP protein is reactivated in a majority of hepatocellular carcinoma (HCC), its expression level was inversely related to the induction of the apoptotic pathway that was chosen as a target of investigation because it is a key mechanism regulating hepatocellular carcinoma growth, is commonly targeted by plant-derived anticancer agents, and comprises discrete, measurable molecular markers (BAX, BCL-2, caspases) that are measurable in stored tissue. Abnormal regulation of apoptosis is involved in tumor survival and resistance, and reconstitution of apoptotic pathways is a proven therapeutic target^{73,74}. In the current study, the DEN group showed a remarked increase in AFP expression compared to other groups. In the post-treated group, there was a noteworthy decrease in AFP expression. TNF- α could be considered an endogenous cancer promoter because it induces cancer cell development, proliferation, invasion, metastasis, and tumor angiogenesis⁷⁵. Nevertheless, it is considered a key inflammatory mediator that triggers immune responses⁷⁰. The serum TNF- α was significantly increased in the DEN group (322.44 ± 11.2) compared to the normal group (0.07 ± 0.02). Besides, the TNF- α in the post-treated group was significantly decreased (73.98 ± 2.7) compared to the DEN group. Histopathological examination of the normal group showed a preserved hepatic lobular architecture with the absence of degeneration, inflammation, or fibrosis. On the other hand, the *H. scoparium* group had a preserved hepatic lobular architecture with focal cholestasis and mild hepatocytic hydropic changes. In the DEN group, there was HCC with a focal acinar formation and bizarre-shaped hyperchromatic nuclei with focal cholestasis and distorted lobular pattern. However, the post-treated group demonstrated focal dysplasia, few apoptotic figures, and cholestasis. IHC results showed a substantial elevation of Caspase-3 expression in

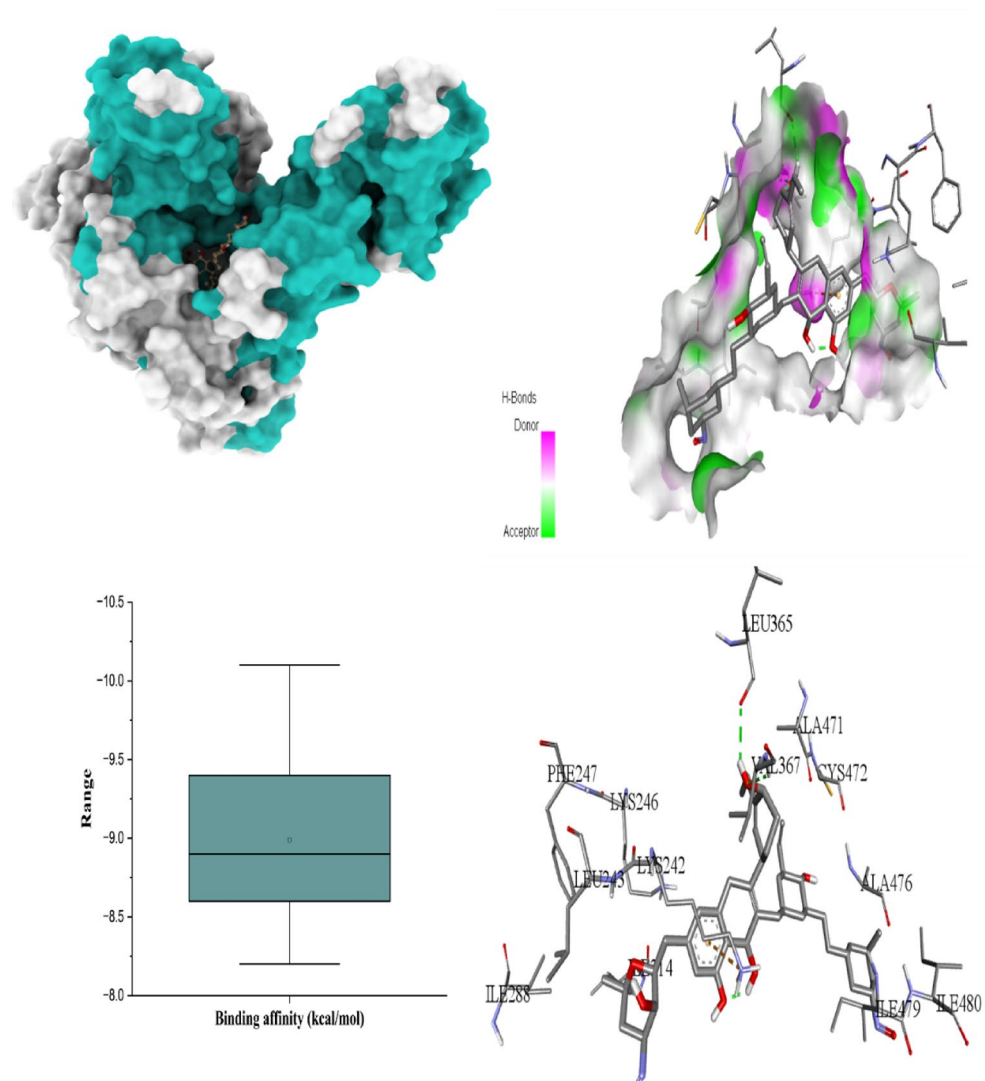


Fig. 11. 3D binding interaction and Box plot of Isorhamnetin triglycoside with AFP.

the DEN/*H. scoparium* group compared to the normal group, which was consistent with qPCR. However, the immunohistochemical analysis of Caspase-3 in the DEN group showed a remarkable increase in the protein expression level that was higher than the treated group (DEN/*H. scoparium*). This result may be attributed to the direct involvement of Caspase-3 in promoting tumor repopulation or a non-apoptotic role for caspases that includes caspase-mediated stimulation of differentiation, dedifferentiation, and T-cell activation⁷⁶. The constitutive phytoconstituents, which were identified in the methanolic extract of *H. scoparium* by LC-MS/MS, displayed significant anticancer properties according to previous research reports. Among all phenolic acid derivatives, ferulic acid-based derivatives had a notable ability in cancer cell proliferation inhibition along with apoptosis induction and oxidative stress manipulation. Laboratory studies displayed its potential cancer-fighting capabilities by controlling tumor advancement through PI3K/Akt and MAPK signaling pathways to become a possible treatment against different cancers⁷⁷. Similarly, the chlorogenic acid derivative, dicaffeoyl quinic acid, exhibited cytotoxic effects against different cancer cells by inducing cell cycle arrest and apoptosis⁷⁸. On the other hand, quercetin and isorhamnetin derivatives were proved to be two significant flavonoidal candidates in the extract. The pro-apoptotic, anti-metastatic, and anti-inflammatory effects of quercetin triglycoside, as a key compound, was explained to promote apoptosis signaling pathways through modulation of NF- κ B and p53, together with caspase activation⁷⁹. Previous reports clarified how are the different isorhamnetin-based glycosides such as tetraglycoside, triglycoside, and rutinoside, exhibit cancer cell antiproliferative and antimigrative properties specifically in lung, liver, and breast cancer cells through their ability to control oncogenic signaling directions, which involve both STAT3 and Wnt/ β -catenin^{80,81}.

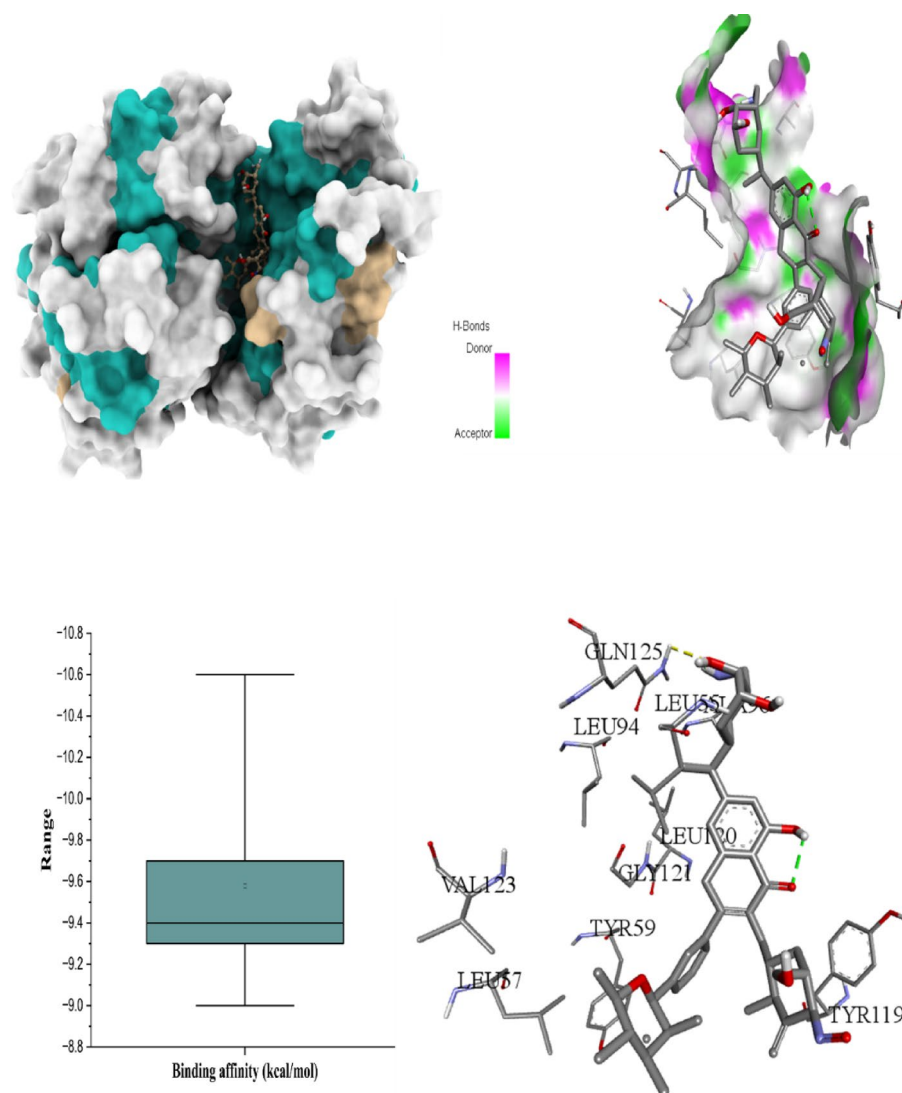


Fig. 12. 3D binding interaction and Box plot of Isorhamnetin triglycoside with TNF- α .

Conclusions

Current findings showed that *H. scoparium* leaf extract protected the liver and fought cancer cells during hepatocellular carcinoma development with DEN exposure. The DEN-induced liver damage was confirmed by elevation of the ALT, AST, ALP, TBILR, AFP, and TNF- α levels and liver tissue changes such as hepatocyte necrosis, together with bile duct proliferation and distorted hepatic structure. The use of *H. scoparium* extract following treatment resulted in both enhanced liver functional parameters and decreased AFP and TNF- α concentrations, while maintaining normal hepatic tissue structure. The anticancer effects of *H. scoparium* extract were associated with enhanced apoptotic activities revealed through elevations in BAX, while Caspase-3 and Caspase-8 expression increased too.

The results validate *H. scoparium* extract's potential function as a preventive and therapeutic solution for HCC. The identification of various compounds in *H. scoparium* recommended its potential as a promising biosafe natural source of anticancer agents. Future studies should focus on isolation such bioactive compounds, elucidating their individual specific mechanisms of action, and exploring their potential for therapeutic applications in cancer treatment.

In silico molecular docking analysis supported the *in vitro* and *in vivo* findings, revealing strong binding affinities for the major glycosylflavonoids, such as quercetin and isorhamnetin glycosides, toward cancer-related proteins, including BCL-2, BAX, Caspase-3, Caspase-8, AFP, and TNF- α . These interactions were stabilized by multiple hydrogen bonds and π - π interactions, especially at key residues involved in apoptotic and oncogenic signaling pathways. Such evidence underscores the multi-target potential of *H. scoparium* phytoconstituents in modulating cancer progression mechanisms.

Limitations and prospects

The hepatoprotective, along with anticancer effects found in this research, need additional consideration for addressing existing limitations. Future investigations should follow pathways of apoptosis together with oxidative stress, inflammation, and angiogenesis to determine the full therapeutic capacity of *H. scoparium*. Long-term chronic exposure research protocols will be useful to determine what sustained effects arise from *H. scoparium* exposure. Future prospective studies should be performed to extend research with *H. scoparium* in liver disease model tests over time to evaluate its ability to stop HCC development. Therapeutic effectiveness and biosafety need additional research regarding *H. scoparium*'s proper dosage levels along with pharmacokinetic testing of its distribution and metabolism patterns, and toxicity evaluation before its clinical use would be approved.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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References

- Boucherit, H. & Benaradj, A. Therapeutic properties of the woody plant *Haloxylon scoparium* Pomel in the steppe region of Naâma (Algeria). *Biodivers. Res. Conserv.* **72**, 39–46 (2024).
- Amtaghri, S. & Eddouks, M. Comprehensive review on the genus *Haloxylon*: Pharmacological and phytochemical properties. *Endocr. Metab. Immune Disord Drug Targets.* **24**, 1146–1160 (2024).
- Purayil, F. T. et al. Transcriptome profiling of *Haloxylon persicum* (Bunge ex Boiss and buhse) an endangered plant species under peg-induced drought stress. *Genes* **11**, 640 (2020).
- Bouaziz, A. et al. Antibacterial and antioxidant activities of *Hammada scoparia* extracts and its major purified alkaloids. *S Afr. J. Bot.* **105**, 89–96 (2016).
- Ebrahim, A. M., Idris, A. M., Alnajjar, A. O. & Michalke, B. Cr and Mn total, accessible species, and protein-fraction contents in plants used for traditional anti-diabetes treatment. *J. Trace Elem. Med. Biol.* **62**, 126645 (2020).
- AL-Sobeai, S. M., Alamer, K. H. & Amer, S. A. M. A preliminary molecular variability within *Haloxylon salicornium* accessions growing in Saudi Arabia. *Plant. Omics.* **8**, 604–608 (2015).
- Bnouham, M., Ziyat, A., Mekhfi, H., Tahri, A. & Legssyer, A. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990–2000). *Int. J. Diabetes Metab.* **14**, 1–25 (2006).
- Lamchouri, F. et al. Preliminary phytochemical and antimicrobial investigations of extracts of *Haloxylon scoparium*. *J. Mater. Environ. Sci.* **3**, (2012).
- Haida, S., Kribii, A., Daoud, N. A., Belakhmima, R. A. & Kribii, A. Antioxidant activity of *Haloxylon scoparium* alkaloid extracts from Figuig region (southeastern of Morocco). *Brazilian J. Pharm. Sci.* **58**, e19494 (2022).
- El-Shazly, A. M., Dora, G. & Wink, M. Alkaloids of *Haloxylon salicornicum* (Moq.) bunge ex Boiss. (Chenopodiaceae). *Pharmazie* **60**, 949–952 (2005).
- Alhawiti, N. M., Alramadhan, W. H., Boadi, W. & Myles, E. L. Anticancer activity of *Peganum harmala* and *Haloxylon salicornicum* leaf extracts on lung cancer A549 and prostate cancer PC-3 cell lines. *Am. J. Plant. Sci.* **14**, 1360–1374 (2023).
- Yang, F. & Lv, G. Combined analysis of transcriptome and metabolome reveals the molecular mechanism and candidate genes of *Haloxylon drought* tolerance. *Front. Plant. Sci.* **13**, 1020367 (2022).
- Yang, F. & Lv, G. Metabolomic analysis of the response of *Haloxylon ammodendron* and *Haloxylon persicum* to drought. *Int. J. Mol. Sci.* **24**, 9099 (2023).
- Guenane, H. et al. Antioxidant properties and mineral contents of different solvent extracts of some medicinal plants cultivated in Algeria. *Trop. J. Nat. Prod. Res.* **8**, 5987–5991 (2024).
- Balogh, J. et al. Hepatocellular carcinoma: a review. *J. Hepatocell Carcinoma.* **3**, 41–53 (2016).
- Okasha, H., Nasr, S. M. & Samir, S. Recombinant expression of Cec-B peptide in *Escherichia coli* with a significant anticancer effect on hepatocellular carcinoma. *Curr. Pharm. Biotechnol.* **22**, 1235–1245 (2021).
- Mohamed, T. S. et al. Chromatographic isolation and characterization of certain bioactive chemical ingredients of *Phyllanthus emblica* extracts and assessment of their potentials as antiviral and anticancer agents. *Egypt. J. Chem.* **65**, 179–192 (2022).
- Kartik, R., Rao, C. V., Trivedi, S. P., Pushpangadan, P. & Reddy, G. D. Amelioration effects against N-nitrosodiethylamine and CCl₄-induced hepatocarcinogenesis in Swiss albino rats by whole plant extract of *Achyranthes aspera*. *Indian J. Pharmacol.* **42**, 370–375 (2010).
- Anisuzzman, M., Hasan, M. M., Acharzo, A. K., Das, A. K. & Rahman, S. *In vivo* and *in vitro* evaluation of pharmacological potentials of secondary bioactive metabolites of *Dalbergia candanensis* leaves. *Evidence-based Complement. Altern. Med.* **2017**, 5034827 (2017).
- Kowsalya, R., Kaliaperumal, J., Vaishnavi, M. & Namasivayam, E. Anticancer activity of *Cynodon dactylon* L. root extract against diethyl nitrosamine induced hepatic carcinoma. *South Asian J. Cancer.* **4**, 83–87 (2015).
- Sarkar, S., Bhattacharjee, P., Ghosh, T. & Bhadra, K. Pharmaceutical efficacy of harmalol in inhibiting hepatocellular carcinoma. *Futur J. Pharm. Sci.* **6**, 29 (2020).
- Thomas, S. et al. *Annona muricata* fruit extract protects against diethylnitrosamine-induced hepatocellular cancer in rats. *Asian Pac. J. Trop. Med.* **12**, 272 (2019).
- Lei, Y. et al. CHOP favors endoplasmic reticulum stress-induced apoptosis in hepatocellular carcinoma cells via Inhibition of autophagy. *PLoS One.* **12**, e0183680 (2017).
- Xu, H., Gao, Y., Shu, Y., Wang, Y. & Shi, Q. EPHA3 enhances macrophage autophagy and apoptosis by disrupting the mTOR signaling pathway in mice with endometriosis. *Biosci. Rep.* **39**, BSR20182274 (2019).
- Ghatei, N. et al. Evaluation of bax, bcl-2, p21 and p53 genes expression variations on cerebellum of BALB/c mice before and after birth under mobile phone radiation exposure. *Iran. J. Basic. Med. Sci.* **20**, 1037 (2017).
- Zhang, H. et al. Diallyl disulfide suppresses inflammatory and oxidative machineries following carrageenan injection-induced paw edema in mice. **2020**, 8508906 (2020).
- Okasha, H., Hassan, M., Aboushousha, T. & Samir, S. Effect of interferon-beta (IFN-β) on tumor suppressor and apoptotic markers in hepatocellular carcinoma cell line. *Int. J. Res. Pharm. Sci.* **10**, 2936–2943 (2019).
- Ghantasala, S. et al. Multiple reaction monitoring-based targeted assays for the validation of protein biomarkers in brain tumors. *Front. Oncol.* **11**, 548243 (2021).
- Ghareeb, M. A. et al. Polyphenolic profile of *Herniaria hemistemon* aerial parts extract and assessment of its anti-cryptosporidiosis in a murine model: *In silico* supported *in vivo* study. *Pharmaceutics* **15**, 415 (2023).

30. Ghareeb, M. A. et al. *Ipomoea carnea* mitigates ethanol-induced ulcers in irradiated rats via Nrf2/HO⁻¹ pathway: an *in vivo* and *in silico* study. *Sci. Rep.* **14**, 3469 (2024).
31. De Melo Silva, A. J., De Melo Gama, J. E. & De Oliveira, S. A. The role of Bcl-2 family proteins and Sorafenib resistance in hepatocellular carcinoma. *Int. J. Cell Biol.* **2024**, 4972523 (2024).
32. Marquardt, J. U. & Edlich, F. Predisposition to apoptosis in hepatocellular carcinoma: from mechanistic insights to therapeutic strategies. *Front. Oncol.* **9**, 1421 (2019).
33. Chen, H. et al. Targeting caspase-8: a new strategy for combating hepatocellular carcinoma. *Front. Immunol.* **15**, 150165 (2024).
34. Mustafa, M. et al. Apoptosis: A comprehensive overview of signaling pathways, morphological changes, and physiological significance and therapeutic implications. *Cells* **13**, 1838 (2024).
35. Porter, A. G. & Jänicke, R. U. Emerging roles of caspase-3 in apoptosis. *Cell. Death Differ.* **6**, 99–104 (1999).
36. Basthi Mohan, P., Lochan, R. & Shetty, S. Biomarker in hepatocellular carcinoma. *Indian J. Surg. Oncol.* **15**, 261–268 (2024).
37. Trott, O. & Olson, A. J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **31**, 455–461 (2010).
38. Amen, Y. et al. Grape-leaf extract attenuates alcohol-induced liver injury via interference with NF-κB signaling pathway. *Biomolecules* **10**, 558 (2020).
39. Elgamal, A. M., Raey, E., Gaara, M. A., Abdelfattah, A., Sobeh, M. & M. A. O. & Phytochemical profiling and anti-aging activities of *Euphorbia retusa* extract: In silico and in vitro studies. *Arab. J. Chem.* **14**, 103159 (2021).
40. Ammar, S. et al. Untargeted metabolite profiling and phytochemical analysis based on RP-HPLC-DAD-QTOF-MS and MS/MS for discovering new bioactive compounds in *Rumex algeriensis* flowers and stems. *Phytochem Anal.* **31**, 616–635 (2020).
41. Kumar, S., Singh, A. & Kumar, B. Identification and characterization of phenolics and terpenoids from ethanolic extracts of *Phyllanthus* species by HPLC-ESI-QTOF-MS/MS. *J. Pharm. Anal.* **7**, 214–222 (2017).
42. Ait Lhaj, Z. et al. Bioactive strawberry fruit (*Arbutus unedo* L.) extract remedies paraquat-induced neurotoxicity in the offspring prenatally exposed rats. *Front. Neurosci.* **17**, 1244603 (2023).
43. Bai, Z. et al. A novel strategy for unveiling spatial distribution pattern of gallotannins in *Paeonia rockii* and *Paeonia ostii* based on LC-QTRAP-MS. *Metabolites* **12**, 326 (2022).
44. Fernández-Ochoa, Á. et al. Metabolite profiling of *Colvillea racemosa* via UPLC-ESI-QTOF-MS analysis in correlation to the *in vitro* antioxidant and cytotoxic potential against A549 non-small cell lung cancer cell line. *Plants* **13**, 976 (2024).
45. Ghareeb, M. A. et al. Chemical profiling of polyphenolics in *Eucalyptus globulus* and evaluation of its hepato-renal protective potential against cyclophosphamide induced toxicity in mice. *Antioxidants* **8**, 415 (2019).
46. Rogozinska, M., Lisiecki, K., Czarnocki, Z. & Biesaga, M. Antioxidant activity of sulfate metabolites of chlorogenic acid. *Appl. Sci.* **13**, 2192 (2023).
47. Świątek, Ł. et al. LC-ESI-QTOF-MS/MS analysis, cytotoxic, antiviral, antioxidant, and enzyme inhibitory properties of four extracts of *Geranium pyrenaicum* Burm. f.: A good gift from the natural treasure. *Int. J. Mol. Sci.* **22**, 7621 (2021).
48. Sobeh, M. et al. A polyphenol-rich extract from *Entada abyssinica* reduces oxidative damage in cryopreserved Ram semen. *Front. Vet. Sci.* **7**, 604477 (2020).
49. Shrivastava, A. K. et al. LC-MS based metabolite profiling, *in-vitro* antioxidant and *in-vivo* antihyperlipidemic activity of *Nigella sativa* extract. *eFood* **4**, e107 (2023).
50. Rodríguez-Pérez, C. et al. Comprehensive metabolite profiling of *Solanum tuberosum* L. (potato) leaves by HPLC-ESI-QTOF-MS. *Food Res. Int.* **112**, 390–399 (2018).
51. Peng, D., Zahid, H. F., Ajlouni, S., Dunshea, F. R. & Suleria, H. A. R. LC-ESI-QTOF/MS profiling of Australian mango peel by-product polyphenols and their potential antioxidant activities. *Processes* **7**, 764 (2019).
52. Pallarés, N. et al. Green and innovative extraction: phenolic profiles and biological activities of underutilized plant extracts using pulsed electric fields and maceration. *Foods* **14**, 222 (2025).
53. Wolff, T. et al. Comprehensive characterisation of polyphenols in leaves and stems of three anti-dengue virus type-2 active Brazilian *Faramea* species (Rubiaceae) by HPLC-DAD-ESI-MS/MS. *Phytochem Anal.* **30**, 62–72 (2019).
54. Kisiriko, M. et al. The first comprehensive chemical profiling of *Vachellia gummifera* (Willd.) Kyal. & Boatwr., a plant with medicinal value. *Chem. Biodivers.* **21**, e202400396 (2024).
55. Ben Salah, H. et al. Flavonol triglycosides from the leaves of *Hammada scoparia* (POMEL) ILJIN. *Chem. Pharm. Bull.* **50**, 1268–1270 (2002).
56. Sobeh, M. et al. *Salix tetrasperma* Roxb. Extract alleviates neuropathic pain in rats via modulation of the NF-κB/TNF-α/NOX/iNOS pathway. *Antioxidants* **8**, 482 (2019).
57. Olennikov, D. N. et al. Bioactive phenolics of the genus *Artemisia* (Asteraceae): HPLC-DAD-ESI-TQ-MS/MS profile of the Siberian species and their inhibitory potential against α-amylase and α-glucosidase. *Front. Pharmacol.* **9**, 756 (2018).
58. Ghareeb, M. A. et al. HPLC-ESI-MS/MS profiling of polyphenolics of a leaf extract from *Alpinia zerumbet* (Zingiberaceae) and its anti-inflammatory, anti-nociceptive, and antipyretic activities *in vivo*. *Molecules* **23**, 3238 (2018).
59. Milutinović, V. et al. Methanol extracts of 28 *Hieracium* species from the Balkan Peninsula-comparative LC-MS analysis, chemosystematic evaluation of their flavonoid and phenolic acid profiles and antioxidant potentials. *Phytochem Anal.* **29**, 30–47 (2018).
60. Qiao, X. et al. Qualitative and quantitative analyses of flavonoids in *Spirodela polyrrhiza* by high-performance liquid chromatography coupled with mass spectrometry. *Phytochem Anal.* **22**, 475–483 (2011).
61. Benkherara, S., Bordjiba, O., Harrat, S. & Djahra, A. B. Antidiabetic potential and chemical constituents of *Haloxylon scoparium* aerial part, an endemic plant from southeastern Algeria. *Int. J. Second Metab.* **8**, 398–413 (2021).
62. Bahri, F. et al. Use of *Haloxylon scoparium* against multidrug-resistant bacteria from urinary tract infections. *Antibiotics* **14**, 471 (2025).
63. Itharat, A. et al. *In vitro* cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. *J. Ethnopharmacol.* **90**, 33–38 (2004).
64. Chelghoum, M., Khitri, W., Benzerga, N. K., Betaouaf, F. Z. K. & Smati, D. Morphological and phytochemical characteristics of *Haloxylon scoparium* Pomel. *Med. Plants* **13**, 627–637 (2021).
65. de Oliveira, P. F. et al. Study of the cytotoxic activity of *Styrax camporum* extract and its chemical markers, egonol and homoegonol. *Cytotechnology* **68**, 1597–1602 (2016).
66. Bailly, C. & Vergoten, G. Anticancer properties and mechanism of action of Oblongifolin C, Guttiferone K and related polyprenylated acylphloroglucinols. *Nat. Prod. Bioprospect.* **11**, 629–641 (2021).
67. Greenwell, M. & Rahman, P. K. S. M. Medicinal plants: their use in anticancer treatment. *Int. J. Pharm. Sci. Res.* **6**, 4103–4112 (2015).
68. Da Rocha, A. B., Lopes, R. M. & Schwartzmann, G. Natural products in anticancer therapy. *Curr. Opin. Pharmacol.* **1**, 364–369 (2001).
69. Tolba, R., Kraus, T., Liedtke, C., Schwarz, M. & Weiskirchen, R. Diethylnitrosamine (DEN)-induced carcinogenic liver injury in mice. *Lab. Anim.* **49**, 59–69 (2015).
70. Wang, H., Liu, J., Hu, X., Liu, S. & He, B. Prognostic and therapeutic values of tumor necrosis factor-α in hepatocellular carcinoma. *Med. Sci. Monit.* **22**, 3694–3704 (2016).
71. Cao, L., Quan, X. B., Zeng, W. J., Yang, X. O. & Wang, M. J. Mechanism of hepatocyte apoptosis. *J. Cell. Death.* **9**, 19–29 (2016).

72. Eimani, B. G. et al. Expression and prognostic significance of Bcl-2 and Bax in the progression and clinical outcome of transitional bladder cell carcinoma. *Cell. J.* **15**, 356–363 (2014).
73. Chen, T. et al. AFP promotes HCC progression by suppressing the HuR-mediated Fas/FADD apoptotic pathway. *Cell. Death Dis.* **11**, 822 (2020).
74. Wang, S. et al. Alpha-fetoprotein inhibits autophagy to promote malignant behaviour in hepatocellular carcinoma cells by activating PI3K/AKT/mTOR signalling. *Cell. Death Dis.* **9**, 1027 (2018).
75. Wang, X. & Lin, Y. Tumor necrosis factor and cancer, buddies or foes? *Acta Pharmacol. Sin.* **29**, 1275–1288 (2008).
76. Huang, Q. et al. Caspase 3-mediated stimulation of tumor cell repopulation during cancer radiotherapy. *Nat. Med.* **17**, 860–866 (2011).
77. Bao, X. et al. Molecular mechanism of ferulic acid and its derivatives in tumor progression. *Pharmacol. Rep.* **75**, 891–906 (2023).
78. Lodise, O. et al. Inhibition of prostate cancer cells by 4,5-dicaffeoylquinic acid through cell cycle arrest. *Prostate Cancer.* **2019**, 4520645 (2019).
79. Özsoy, S., Soykut, G. & Becer, E. Quercetin: A phytochemical with pro-apoptotic effects in colon cancer cells. *Cyprus J. Med. Sci.* **7**, 587–592 (2022).
80. Li, C., Yang, X., Chen, C., Cai, S. & Hu, J. Isorhamnetin suppresses colon cancer cell growth through the PI3K-Akt-mTOR pathway. *Mol. Med. Rep.* **9**, 935–940 (2014).
81. Wei, J. et al. Anti-proliferative effect of Isorhamnetin on HeLa cells through inducing G2/M cell cycle arrest. *Exp. Ther. Med.* **15**, 3917–3923 (2018).

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Conceptualization, M.A.G., B.B., M.M., and H.O.; methodology, M.A.G., B.B., Y.A., T.A., and H.O.; software, M.A.G., Y.A., T.A., and H.O.; validation, M.A.G., B.B., M.M., and H.O.; formal analysis, M.A.G., B.B., Y.A., T.A., and H.O.; investigation, M.A.G., B.B., Y.A., T.A., M.M., and H.O.; resources, M.A.G., and H.O.; data curation, M.A.G., Y.A., T.A., and H.O.; writing—original draft preparation, M.A.G., Y.A., T.A., and H.O.; writing—review and editing, M.A.G., B.B., T.A., M.M., and H.O.; visualization, M.A.G., B.B., Y.A., M.M., and H.O.; supervision, M.A.G., T.A., M.M., and H.O.; project administration, M.A.G., and H.O.; funding acquisition, M.A.G., and H.O. All authors have read and agreed to the published version of the manuscript.

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Declarations

Compliance with ethical standards

All procedures in this study, including handling, anesthesia, and sacrifice, adhered to the ethical guidelines approved by the Ethical Committee of the Federal Legislation and the National Institutes of Health Guidelines in the United States. All animal experiments were carried out under Institutional Ethical Committee rules for the care and use of experimental animals, which were authorized by Theodor Bilharz Research Institute's Animal Ethics Committee in Giza, Egypt (FWA 00010609). All methods are documented in line with the ARRIVE guidelines for reporting animal experiments. (<https://arriveguidelines.org>).

Competing interests

The authors declare no competing interests.

Additional information

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