RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 5: 769-776 (May 2025)

Phytochemical Profiling and Cytotoxic Potential of *Senecio flavus* n-Hexane Extract using *in vitro* and *in silico* Approaches

N. Abutaha[1], B.S.A. Al-Dawsari[1], S. Al-Qahtani[1], A.M. Rady[1], F.A. Al-Mekhlafi[1]

10.18805/IJAR.BF-1843

ABSTRACT

Background: Using natural products as potential anticancer and antimicrobial agents has gained significant attention.

Methods: This study investigated the anticancer and antimicrobial properties of *Senecio flavus* by using MTT and well-diffusion assays, respectively. The study also examined the potential of *S. flavus* extracts to induce apoptosis in liver cancer cells using light and fluorescent microscopy. Gas Chromatography-Mass Spectrometry (GC-MS) was utilized to identify the phyto-compounds, which were subsequently analyzed through molecular docking using AutoDock Vina 1.1.2.

Result: The difference in solvent polarity and extraction methods had a notable influence on the yield of the extracts. The saponin fraction had the highest yield (4.42%). Only the hexane extract demonstrated cytotoxic with IC_{50} values of 241.2 and 187.8 µg/mL for HepG2 and Huh-7 cells, respectively. Apoptotic morphological changes were observed in cells treated with the hexane extract. Additionally, apoptotic cell induction was confirmed using DAPI and AO-EB dual staining. The saponin fraction of *S. flavus* demonstrated antimicrobial activity, indicated by inhibition zones measuring 19 mm, 27 mm, 13 mm, 17 mm 12 mm against *Acinetobacter baumannii* (MDR), *Acinetobacter baumannii* (ATCC® BAA-747), *Staphylococcus epidermidis*, *Staphylococcus aureus Listeria monocytogenes*, respectively. GC-MS analysis identified a range of bioactive phyto-compounds within the hexane extract. The compounds (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho[2,3-b]furan-4(6H)-one and Tricyclo [20.8.0.0 (7,16)]triacontane, 1(22),7(16)-diepoxy- demonstrated the most favourable docking results with 4JAS and 5W1B, respectively, exhibiting a ΔG of -9.7 kcal/mol. This highlights the potential of *S. flavus* as a promising plant, which requires further investigation and development as a therapeutic agent.

Key words: *Acinetobacter baumannii*, Apoptosis, GC-MS, Liver cancer, Saponin, *Senecio flavus*.

INTRODUCTION

Liver cancer is an aggressive type of cancer with a high fatality rate (Siegel *et al*., 2018). Chemotherapy offers limited benefits to a minority of patients, often leading to drug-related toxicity or inefficacy over time. Although surgical interventions are available, the prognosis remains unfavourable due to frequent relapses (Liang *et al*., 2021). Treatment with sorafenib is widely used in late-stage cases and presents significant challenges. Less than a third of patients experience any benefit from this therapy resistance to the drug typically develops within six months of starting treatment (El-Serag *et al.,* 2008).

Moreover, prolonged use of chemotherapeutic agents like sorafenib is associated with toxicity issues and decreased effectiveness. Consequently, existing chemotherapy has failed to yield substantial improvements for liver cancer patients. Therefore, there is a need for extensive research to identify more effective approaches for managing this disease (Anwanwan *et al*., 2020). Similarly, the widespread misuse of antibiotics resulted in increased antimicrobial resistance, rendering many antibiotics ineffective (Vaou *et al*., 2021). The World Health Organization (WHO, 2023) considers this alarming trend a critical issue, highlighting the urgent need for novel antimicrobial agents. These new agents must be capable of reducing antibiotic usage and combating the development of resistance.

Traditional plants are being assessed as an alternate therapeutic approach to overcome the limits of anticancer and antimicrobial current treatments (Dehghani and Saeidi 2023; Alghamdi *et al*., 2024; Moatasem *et al*., 2023). *Senecio flavus* (Decne.) is a member of the Asteraceae family and is found in Afghanistan, Algeria, Spain, Chad, Egypt, Iran, Mauritania, Morocco, Namibia, Pakistan, Palestine, Sinai, Spain, Sudan, Tunisia (https://www.gbif.org/species/3107479). However, there is a significant gap in scientific research on *S. flavus* and its potential therapeutic efficacy.

GC-MS is extensively employed to identify bioactive phytocompounds. It is a highly effective, fast accurate method for detecting various chemicals such as nitro compounds, amino acids, alkaloids, alcohols, organic acids, steroids esters long-chain hydrocarbons (Al-Rubaye *et al*., 2017). Similarly, computer-based tools have emerged as advanced strategies in drug discovery, particularly in

[1]Department of Zoology, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia.

Corresponding Author: N. Abutaha, Department of Zoology, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia. Email: nabutaha@ksu.edu.sa

How to cite this article: Abutaha, N., Al-Dawsari, B.S.A., Al-Qahtani, S., Rady, A.M. and Al-Mekhlafi, F.A. (2025). Phytochemical Profiling and Cytotoxic Potential of *Senecio flavus* n-Hexane Extract using *in vitro* and *in silico* Approaches. Indian Journal of Animal Research. 59(5): 769-776. doi: 10.18805/IJAR.BF-1843.

Submitted: 06-07-2024 **Accepted:** 18-10-2024 **Online:** 09-12-2024

screening medicinal plants for bioactive compounds. Molecular docking, characterized by its efficiency and cost-effectiveness, is a valuable approach for developing and testing pharmaceuticals. This technique provides insights into drug-receptor interactions, enabling predictions on how drug models will bind to target proteins, ultimately leading to reliable binding at specific ligand binding sites (Pagadala *et al*., 2017).

Therefore, this study evaluated the antimicrobial and cytotoxic activities of various extraction methods of *S. flavus*, identified potential bioactive components using Gas Chromatography-Mass Spectrometry (GC/MS) employed in-silico methods for further analysis.

MATERIALS AND METHODS

Study area

This study was carried out at King Saud University, Diriyah, Saudi Arabia, from March 2022 to February 2024

Collection of the plant

The *Senecio flavus* (Decne.) was collected from Riyadh, King Saud University campus. The plant was authenticated in the Department of Botany (voucher specimen: KSU-RYD-091) and was preserved in the Bioproduct Research Chair at King Saud University. The aerial part of the *S. flavus* was washed with distilled water, shade-dried, pulverized stored in an airtight container (Fig 1).

Extraction and fractionation

Seventy-two grams of powdered material was macerated in 500 mL of 70% methanol for 3 days at 25°C with occasional shaking. Subsequently, the mixture was filtered through a muslin cloth the resulting extract was evaporated at 45°C using a rotary evaporator. The concentrate was then suspended in 500 mL of distilled water and subjected to liquid-liquid extraction (2×200 mL) using hexane (F1), ethyl acetate (F2) n-butanol (F3), respectively. The aqueous layer was discarded each separated fraction was concentrated individually using a rotary evaporator (Sadiq *et al*., 2014).

Saponin extraction

To extract the sample, 20 grams were placed in a glass flask and mixed with 500 mL of 15% aqueous ethanol. The mixture was heated using a hot water bath with continuous stirring at 55°C for 4 hours. Afterwards, the extract was rotary evaporated to 50 mL at 50°C and then transferred into a 500 mL separatory funnel. To prepare the extract, petroleum ether (2×200 mL) was added and vigorously shaken. The resulting aqueous layer was retained, whereas the ether layer was discarded. This purification procedure was repeated n-butanol (2×200 mL) was added. The combined n-butanol extract underwent washing with 100 mL of 5% aqueous NaCl, repeated twice. Subsequently, the remaining solution was evaporated (Edeoga *et al*., 2005).

Cell culture

The HepG2 and Huh-7 liver cancer cell lines were obtained from the German cell culture collection (DSMZ) and maintained as monolayers in 75 cm^2 flasks (Nest, China) using Dulbecco's Modified Eagle's Medium (DMEM). The DMEM media (Invitrogen, USA) were supplemented with 10% fetal bovine serum (Invitrogen, USA). The cells were cultured under at 37°C in a humidified atmosphere with 5% CO_2.

Cytotoxicity assay

The cells (25,000 cells/well) were seeded in 48-well plates (Nest, China) and incubated for 24 hours at 37°C in a humidified atmosphere of 5% CO_2. Following this incubation period, the cells were treated with varying extract concentrations (500-25 μg/mL) for 24 hours. Subsequently, 50 μL of MTT solution (Invitrogen, USA) was added to each well the plates were incubated at 37°C for 2 h. The optical densities were measured at a wavelength of 490 nm using a multi-well plate reader (ChroMate, USA). The results are



Fig 1: A fresh and air-dried aerial part of *Senecio flavus* collected from Riyadh, Saudi Arabia.

presented as a percentage of the control and were used to calculate the inhibitory concentration (IC_{50}) values using OriginPro 8.5. The IC_{50} value represents the extract concentration required to reduce the cell number to 50% of the untreated control. Each extract was tested in triplicate (Khasawneh *et al.,* 2022).

Analysis of cell morphology

As reported earlier, human liver cancer cells (Huh-7) were seeded in 48-well plates and incubated for 24 hours to facilitate cell attachment and growth. Post-incubation, the cells were exposed to *S. flavus* extract at a concentration of 150 µg/mL, with the hexane extract prepared in DMSO ensuring that the final DMSO concentration in each well did not exceed 0.1%. The plates were incubated for an additional 24 h under identical conditions (37°C, 5% CO_2). Following the 24-hour treatment period, the cell morphologies in treated and untreated control wells were observed and recorded using an inverted microscope (EVOS, USA).

Fluorescence staining of cells

The cells were seeded, treated with the hexane extract (150 µg/mL) incubated for 24 h as described in the previous section (Analysis of Cell Morphology). After incubation, the cells were stained with a 42 ,6-diamidino-2-phenylindole (DAPI) solution at a 5 µg/mL concentration. Similarly, for Acridine Orange/Ethidium Bromide (AO/EB) staining (Sigma-Aldrich, USA), the cells were stained with a solution containing 100 µg/mL AO and 100 µg/mL EB in PBS for 2 minutes. The stained cells were then processed according to the method reported by (Khasawneh *et al*., 2022). Images were captured using an EVOS fluorescent microscope.

Microbial strains and culture

The microbial strains required for the study were sourced from the Microbiology Department at King Saud University in Riyadh, Saudi Arabia. Six of the selected microbial species were bacterial strains; one was a fungal strain investigated for its antifungal properties. The bacterial strains, including *Acinetobacter baumannii* (ATCC®BAA-747), *Staphylococcus epidermidis* (clinical isolate), *Bacillus subtilis* 16406, *Staphylococcus aureus* (ATCC®29213) *Listeria monocytogenes* (ATCC®7644) were subcultured on Nutrient Agar (NA) media. These cultures were grown at 37°C for 24 hours. Meanwhile, the fungal strain *C. albican* (ATCC® 90028) was subcultured in potato dextrose agar.

Antimicrobial assay of extracts

The antimicrobial activity of the extracts was evaluated using the agar well diffusion method on Nutrient Agar (HiMedia, India) for bacterial strains and Potato Dextrose Agar (HiMedia, India) for fungal strains. The test organisms were inoculated into broth media the turbidity was adjusted to an OD_{600} of 0.01 to standardize the inoculum density. Plant extracts were prepared in methanol (MeOH) at 50 mg/mL. Using a sterile cork borer (6 mm), wells were created in the inoculated media plates 25 µL of each extract was pipetted into the wells. The plates were allowed to sit at room temperature (25°C) for 30 minutes to facilitate diffusion of the extracts into the agar. Subsequently, the plates were incubated at 37°C for 24 h for bacterial cultures and at 30°C for 24 hours for fungal cultures. Antibiotic tetracycline discs (30 µg) from Sigma Aldrich, Germany, were used as positive controls, while methanol (MeOH) was the negative control. Following incubation, the plates were examined for clear inhibition zones around the wells, indicating antimicrobial activity. The diameter of the inhibition zones was measured in millimetres using a digital calliper.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis followed the protocol outlined by (Abd El-Kareem *et al*., 2016). The chemical composition of hexane extract was assessed using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a TG-5MS capillary column (30 m×0.25 mm×0.25 µm film thickness). Compounds were identified by referencing the NIST14 and WILEY 09 mass spectral databases.

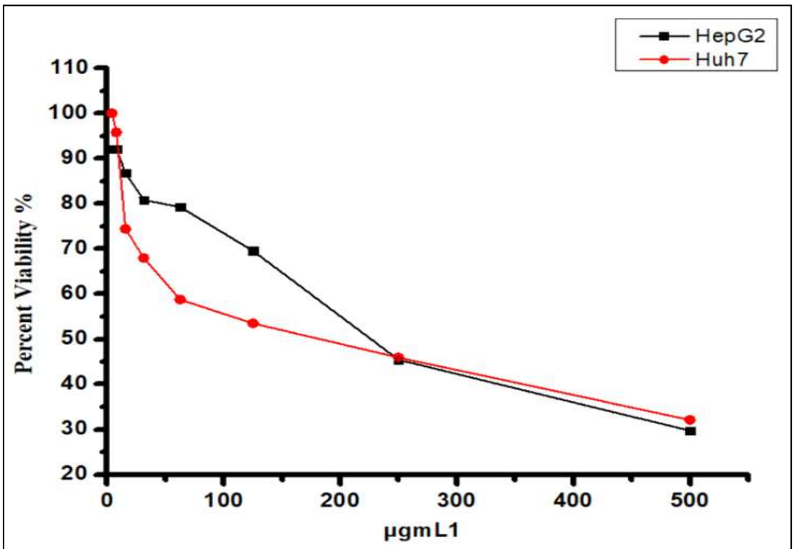
Molecular docking

X-ray structures of sixteen target receptors (3FV7), (3TD4), (3ZNT), (4FUV), (4JAS), (4JF4), (4KOX), (4KOV), (4OHO), (4QDI), (4X55), (Y0A), (5BUF), (5W1B), (6FJY) and (6GIE) were downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org). Thirty-four phyto-compounds were obtained from PubChem as structure data files (SDF) and converted to '.pdb' format using Open Babel 2.3.2. Autodock 4.2 was then utilized to generate the 'pdbqt' file of the ligands. The protein structures were prepared for docking using AutoDockTool-1.5.7 (Huey *et al.,* 2007), calculating Gasteiger charges, removing water molecules adding polar hydrogens and site-specific docking grid coordinates (Morris *et al.,* 2009). Molecular docking procedures were carried out using AutoDock Vina 1.1.2. The resulting conformations of the ligand-receptor complexes were ranked based on their binding energy.

Results and Discussion

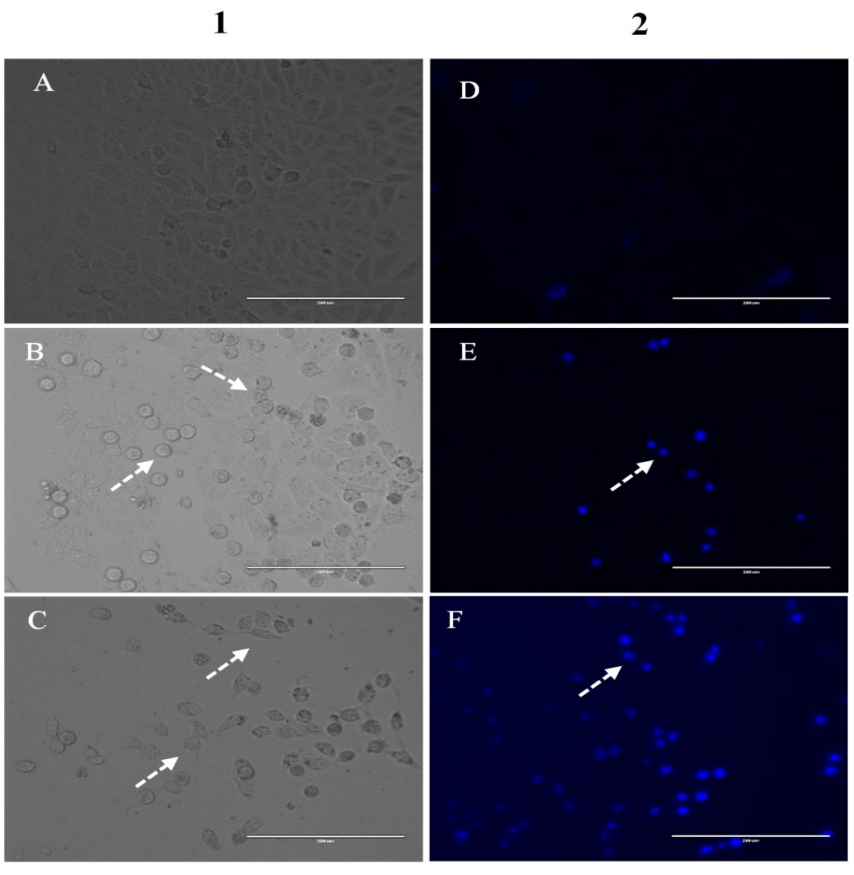
This study evaluated the yield of extracts from *S. flavus* using various extraction techniques. The saponin fraction yielded the highest percentage, comprising 4.42% of the total extract. This was followed by the n-butanol extract at 0.79%, the n-hexane extract at 0.54% the ethyl acetate extract at 0.36%.

Two liver cancer cell lines were chosen to assess the cytotoxicity using the MTT assay. The results revealed that only the hexane extract exhibited cytotoxic effects on the tested cell lines, whereas the other extracts showed more than 96% viability at the highest concentration tested (500 µg/mL). The IC_{50} values at 24 h post-treatment in HepG2 and HuH-7 cells were 241.2 and 187.8 µg/mL, respectively. These findings indicate that the hexane extract possesses higher cytotoxicity in HuH-7 cells than in HepG2 cells (Fig 2).



Each value represents mean±SD of three independent experiments.

Fig 2: Cytotoxicity of hexane extract of *Senecio flavus* against HepG2 and HuH-7 cells *via* MTT assay.



(1) and the nuclear morphology of Huh-7 cells stained with DAPI (2). A and D: Control sample treated with 0.01% (v/v) MeOH at 24 h. B, C, E and F: cells treated with hexane extract at 50 and 250 µg/mL respectively at 24 h.

Fig 3: The effect of hexane extract on cell morphology.

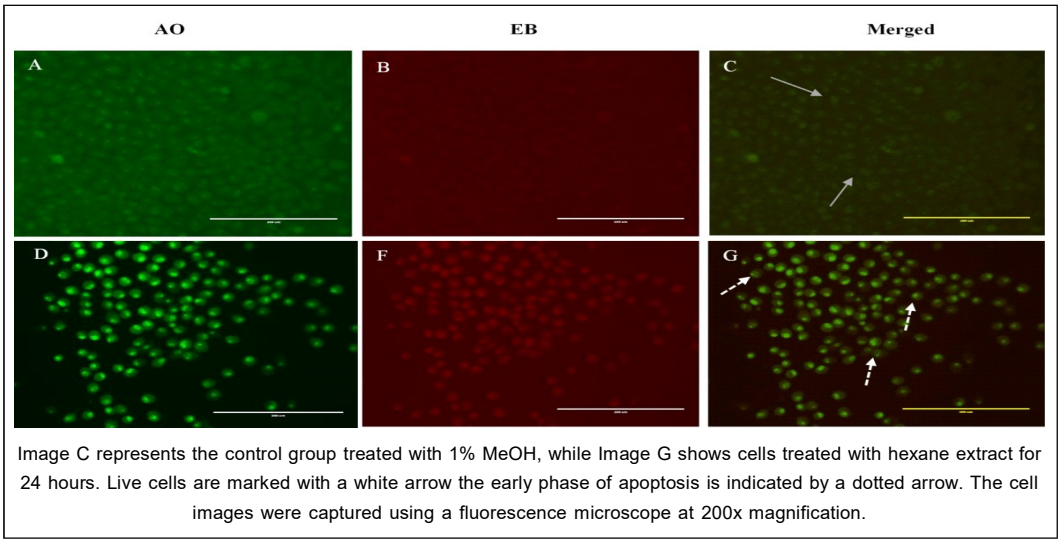


Image C represents the control group treated with 1% MeOH, while Image G shows cells treated with hexane extract for 24 hours. Live cells are marked with a white arrow the early phase of apoptosis is indicated by a dotted arrow. The cell images were captured using a fluorescence microscope at 200x magnification.

Fig 4: Acridine orange/ethidium bromide dual staining was employed to observe the apoptotic features in Huh-7 cells.

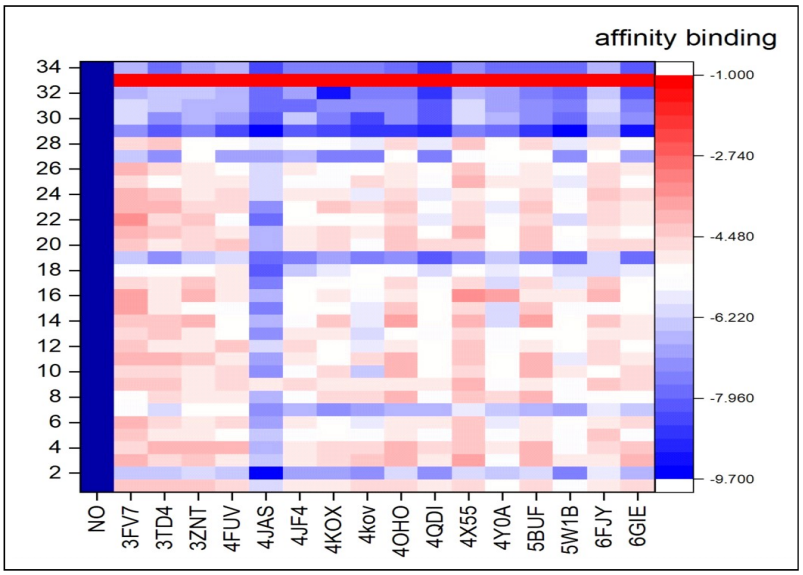


Fig 5: Heatmap of molecular docking scores of thirty-four ligands and sixteen target proteins.

Table 1: Inhibition zone (in mm) of saponin fraction of *Senecio flavus* against drug resistant and clinical microbes used in antimicrobial testing.

Microorganism	Origin	Resistance phenotype	Means of bacterial growth inhibition zones	
Acinetobacter baumannii 1100	Clinical isolate		Saponin	Tetracycline
		Amoxicillin, Ampicillin, Aztreonam, Cefepime, Cefoxitin, Ceftazidime, Cefuroxime Cephaloxime, Ciprofloxacin, Gentamicin, Imipenem, Meropenem, Nitrofurantoin, Tigecycline,	19±0.35	0.0±0.0
Acinetobacter baumannii	ATCC®BAA-747	-	27±0.25	22±0.0
Staphylococcus epidermidis	Clinical isolate	-	13±0.55	20±0.15
Staphylococcus aureus	ATCC®29213	-	17±0.55	24±0.35
Listeria monocytogenes	ATCC® 7644	-	12±0.95	38±0.15
Candida albicans	ATCC®90028	-	-	-

Table 2: Major phyto-compounds were identified in the hexane extract of *Senecio flavus*.

Retention time	Name of the compound	Area %	Molecular formula	Molecular weight	Description
18.27	Dodecanoic acid	0.55	$C_{12}H_{24}O_2$	200	Fatty acid
23.77	(R)-3,5,8a-Trimethyl-7,8,8a,9-tetrah ydronaphtho[2,3-b]furan-4(6H)-one	0.36	$C_{15}H_{18}O_2$	230	Ketone
25.62	Palmitic acid methyl ester	0.32	$C_{17}H_{34}O_2$	270	Fatty acid
26.56	Hexadecanoic acid	4.34	$C_{16}H_{32}O_2$	256	Fatty acid
28.63	9,12-Octadecadienoic acid, methylester, (E,E)	1.10	$C_{19}H_{34}O_2$	294	ester
28.82	9-Octadecenoic acid, methyl ester, (E)-	1.67	$C_{19}H_{36}O_2$	296	Ester
28.94	5-Benzofuranacetic acid, 6-ethenyl -2,4,5,6,7,7a-hexahydro-3,6	0.52	$C_{16}H_{20}O_4$	276	-
29.16	Ferrocene, 1,1'-[1,2-Ethanediylbis (THIO)]	5.0	$C_{14}H_{12}O_6$	276	Phenolic compound with sulfur Substitutions.
30.06	cis-13-Octadecenoic acid	69.18	$C_{18}H_{34}O_2$	282	Fatty acid
30.31	Oleic acid	0.26	$C_{18}H_{34}O_2$	282	Fatty acid
32.05	Glycidyl oleate	0.60	$C_{21}H_{38}O_3$	338	Fatty acid
32.29	9-Octadecenoic acid (Z)-	0.27	$C_{18}H_{34}O_2$	282	Fatty acid
32.45	6-Octadecenoic acid	0.74	$C_{18}H_{34}O_2$	282	Fatty acid
32.90	trans-13-Octadecenoic acid	0.33	$C_{18}H_{34}O_2$	282	Fatty acid
34.71	9,12,15-Octadecatrienoic acid,	1.51	$C_{21}H_{36}O_4$	352	Fatty acid
34.85	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl Ester	1.87	$C_{21}H_{38}O_3$	338	Ester
35.34	Hexadecanoic acid, 2,3-dihydroxypropyl ester	0.52	$C_{19}H_{38}O_4$	330	Ester
35.82	Diisooctyl phthalate	1.24	$C_{24}H_{38}O_4$	390	Ester
36.29	Phorbol	0.26	$C_{20}H_{28}O_4$	364	Terpene
36.41	Ethanol, 2-(9-octadecenyloxy)-, (Z)	0.22	$C_{20}H_{40}O_2$	312	
36.55	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	0.23	$C_{20}H_{40}O_2$	312	Ester
37.23	2-HYDROXY-3-[(9E)-9-OCTADEC ENOYLOXY]PROPYL	0.40	$C_{39}H_{72}O_5$	620	Ester
37.52	Linoleic acid ethyl ester	0.34	$C_{20}H_{36}O_2$	308	Fatty acid ester
37.61	9-Octadecenoic acid (Z)-, 2-hydroxy -1-(hydroxymethyl)ethylEster	0.58	$C_{21}H_{40}O_4$	356	Ester
37.95	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethylEster	2.28	$C_{21}H_{38}O_4$	354	Fatty acid ester
38.43	Oleic Acid	0.86	$C_{18}H_{34}O_2$	282	Fatty acid
40.15	2,2,4-Trimethyl-3-(3,8,12,16-tetrame thyl-heptadeca-3,7,11,15-tetraenyl)-c	0.29	$C_{30}H_{52}O$	428	-
42.54	9,12,15-octadecatrienoicAcid	0.23	$C_{27}H_{52}O_4Si_2$	496	Fatty acid
42.64	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	0.54	$C_{27}H_{30}O_{16}$	610	Flavonoids
43.05	4H-1-BENZOPYRAN-4-ONE, 2-(3,4-DIMETHOXYPHENYL)-3,5-DIHYDROXY-7-METHOXY-	0.31	$C_{18}H_{16}O_7$	344	Sesquiterpn lactone
43.92	Ethyl iso-allocholate	0.27	$C_{26}H_{44}O_5$	436	Steroid
44.22	Stigmasterol	0.36	$C_{29}H_{48}O$	412	Sterol
44.74	STIGMAST-5-EN-3-OL, (3á,24S)	1.72	$C_{29}H_{50}O$	414	Sterol
45.0	4H-1-BENZOPYRAN-4-ONE, 2-(3,4-DIHYDROXYPHENYL)-6,8-DI-á-D-GLUCOPYRANOSYL-5,7- DIHYDROXY	0.66	$C_{27}H_{30}O_{16}$	610	Flavonoid glycoside

In addition, *S. flavus* extracts were studied for their potential to induce apoptosis in liver cancer cells. Apoptosis is defined by morphological characteristics such as detachment, cell shrinkage, cell rounding nuclear chromatin condensation (Doonan and Cotter, 2008). Our findings revealed that treatment with hexane extract led to noticeable morphological changes. In Fig 3A and 4A, the cells in the control wells exhibited attachment to the surface, reaching 95-100% confluence and maintaining their typical shape.

Similarly, upon staining with DAPI, as shown in Fig 3A and 4A for control samples, cell nuclei appeared uniform in shape and size. In contrast, the nuclei of samples treated with the hexane extract, as depicted in Fig 3B, 3C, 4B 4C, showed signs of shrinkage, condensation fragmentation in areas with abnormal cells. These changes strongly support that n-hexane extract activated apoptotic deaths in liver cancer cells. AO-EB dual staining was carried out to support our findings. We observed that the viable control HuH-7 cells had evenly distributed green fluorescence (AO stain), normal nuclear morphology no red fluorescence (Fig 5A). In contrast, cell viability significantly decreased in n-hexane extract-treated HuH-7 cells. However, the number of apoptotic cells increased, as is evident from the bright green and yellow-orange staining resulting from chromatin condensation and membrane integrity loss (Fig 6). Apoptosis induction is considered one of the most effective strategies for chemotherapy when developing new anticancer agents (Surh, 2003). Therefore, identifying candidate anticancer agents that can induce apoptosis in cancer cells is crucial in the search for new and effective anticancer treatments.

The most challenging aspect of microbial infections is multidrug resistance (MDR), a critical concern where conventional antibiotic treatments are often ineffective. The search for novel and potent antibacterial agents targeting MDR-*A. baumannii* is of utmost importance (Rafailidis *et al.,* 2024). In current study, only the saponin fraction exhibited antimicrobial activity, as evidenced by inhibition zones with diameter values of 19 mm, 27 mm, 13 mm, 17 mm 12 mm against *Acinetobacter baumannii* 1100 (MDR), *A. baumannii* (ATCC®BAA-747), *S. epidermidis, S. aureus L. monocytogenes*, respectively (Table1). However, none of the extracts showed activity against *C. albicans*. Other researchers have reported similar results using different plant extracts. Researchers found that the oil extracted from basil, clove cinnamon exhibited potent bactericidal activity against MDR-*A. baumannii*, with minimum bactericidal concentrations (MBC_{90}) of 2,1 and 0.5 mg/mL, respectively (Intorasoot *et al*., 2017). Our results also revealed that the saponin fraction was not toxic to the cell lines tested up to 1 mg/mL, indicating the safe use of the extract on human liver cells. However, further experiments are needed to assess its toxicity. Makhafola *et al.* (2012) emphasized that natural products should not be considered safe until they undergo cellular toxicity tests. It is crucial to assess the cytotoxicity using multiple cell lines because relying on just one cell line to establish the safety and efficacy of an extract can be misleading. The sensitivity of cell lines is expected to vary due to differences in metabolic activities and uptake capabilities (Froscio *et al*., 2009).

A GC-MS analysis was conducted to determine the quantitative analysis of hexane extract of *S. flavus*. A total of 34 bioactive phyto-compounds were identified using GC-MS analysis (Table 2). The major identified compound was cis-13-Octadecenoic acid (69.1%) followed by hexadecanoic acid (4.34%). The GC-MS results of the hexane extract from *S. flavus* revealed the existence of potential antitumor and antimicrobial composites. For example, 9-octadecenoic acid (z)-, methyl ester and 9-Octadecenoic acid (Z)- have also been reported to have antioxidant, anticancer antimicrobial potential (Duke, 1994) (Diab *et al*., 2021). However, 9,12-octadecadienoic acid methyl ester has also been reported to have antitumor and antioxidant properties (Reza *et al.,* 2021).

The selected drug-like molecules of *S. flavus* were docked with sixteen different target receptors, namely 3FV7, 3TD4, 3ZNT, 4FUV, 4JAS, 4JF4, 4KOX, 4KOV, 4OHO, 4QDI, 4X55, 4Y0A, 5BUF, 5W1B, 6FJY and 6GIE (Fig 5). Compounds 2 and 29 were the best docked to 4JAS and 5W1B with ÄG of -9.7 kcal/mol for both compounds.

Conclusion

The findings from this study reveal that hexane extract showed promising cytotoxic and apoptotic effects against HepG2 and Huh-7. The saponin fraction exhibited antimicrobial activity against *A. baumannii*, indicating its potential as an antimicrobial agent. GC-MS analysis revealed a range of bioactive compounds in the hexane extract, with (R)-3,5,8a-Trimethyl-7,8,8a,9-tetrahydronaphtho [2,3-b] furan-4(6H)-one and Tricyclo [20.8.0.0 (7,16)] triacontane, 1(22),7(16)-diepoxy- showing favourable interactions in molecular docking studies. Further research is needed to explore its potential for medical applications.

Acknowledgement

The present study was supported by Researchers Supporting Project number (RSP2024R112), King Saud University, Riyadh, Saudi Arabia.

Funding

This project was funded by Researchers Supporting Project number (RSP2024R112), King Saud University, Riyadh, Saudi Arabia.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Abd El-Kareem, M.S., Rabbih, M.A.E.F., Selim, E.T.M., Elsherbiny, E.A.E., El-Khateeb, A.Y. (2016). Application of GC/EIMS in combination with semi-empirical calculations for identification and investigation of some volatile components in basil essential oil. International Journal of Analytical Mass Spectrometry and Chromatography. 4: 14-25.

Al-Rubaye, A.F., Hameed, I.H., Kadhim, M.J. (2017). A review: Uses of gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants. International Journal of Toxicological and Pharmacological Research. 9: 81-85.

Alghamdi, R., Abutaha, N., Almekhlafi, F.A. and Wadaan, M.A. (2024). Metabolic Profiling, *in vitro* Cytotoxicity *in silico* Investigation of *Lycium shawii* Roem. Extract. Indian Journal of Animal Research. 58(9). doi: 10.18805/IJAR.BF-1751.

Anwanwan, D., Singh, S.K., Singh, S., Saikam, V. Singh, R. (2020). Challenges in liver cancer and possible treatment approaches. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 1873: 188314.

Dehghani, M. Saeidi, S. (2023). Antimicrobial effects of medicinal plant species on salmonella typhimurium strains isolated from poultry feces samples. Jentashapir Journal of Cellular and Molecular Biology. 14(4): e135947. https://doi.org/10.5812/jjcmb-135947.

Diab, T.A., Donia, T. Saad-Allah, K.M. (2021). Characterization, antioxidant cytotoxic effects of some Egyptian wild plant extracts. Beni-Suef University Journal of Basic and Applied Sciences. 10: 1-13.

Doonan, F. Cotter, T.G. (2008). Morphological assessment of apoptosis. Methods. 44(3): 200-204.

Duke, J.A. (1994). Dr. Duke's Phytochemical and Ethnobotanical Databases.

Edeoga, H.O., Okwu, D. Mbaebie, B. (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 4: 685-688.

El-Serag, H.B., Marrero, J.A., Rudolph, L., Reddy, K.R. (2008). Diagnosis and treatment of hepatocellular carcinoma. Gastroenterology. 134: 1752-1763.

Froscio, S.M., Fanok, S., Humpage, A.R. (2009). Cytotoxicity screening for the cyanobacterial toxin cylindrospermopsin. Journal of Toxicology and Environmental Health, Part A. 72: 345-349.

Huey, R., Morris, G.M., Olson, A.J. Goodsell, D.S. (2007). A semiempirical free energy force field with charge based desolvation. Journal of Computational Chemistry. 28: 1145-1152.

Intorasoot, A., Chornchoem, P., Sookkhee, S. Intorasoot, S. (2017). Bactericidal activity of herbal volatile oil extracts against multidrug-resistant Acinetobacter baumannii. Journal of Intercultural Ethnopharmacology. 6(2): 218-222.

Khasawneh, M.A., AlKaabi, A., Samadi, A., Antony, P., Vijayan, R., Al-Keridis, L.A., Saadeh, H.A. Abutaha, N. (2022). Synthesis and biological applications of some novel 8-Hydroxyquinoline urea and thiourea derivatives. Arabian Journal of Chemistry. 15: 103905.

Liang, F., Ma, F. Zhong, J. (2021). Prognostic factors of patients after liver cancer surgery: Based on surveillance, epidemiology end results database. Medicine. 100(30): e26694.

Makhafola, T.J., Samuel, B.B., Elgorashi, E.E. Eloff, J.N. (2012). Ochnaflavone and ochnaflavone 7-O-methyl ether two antibacterial biflavonoids from *Ochna pretoriensis* (Ochnaceae). Natural Product Communications. 7(12): 1934578X1200701216.

Moatasem, M.A., Dhifaf, J.S., Emaduldeen, H.A., Mohammed, M.T.A., Ibrahim, S.A., Dhuha, J.M., and Rana, R.K. (2023). The synergistic anticancer effect of some plant extracts in combination against human liver cancer cell line. Asian Journal of Dairy and Food Research. 42: 529-534. doi: 10.18805/ajdfr.DRF-297.

Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S. Olson, A.J. (2009). AutoDock4 and Auto DockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry. 30: 2785-2791.

Pagadala, N.S., Syed, K. Tuszynski, J. (2017). Software for molecular docking: A review. Biophysical Reviews. 9: 91-102.

Rafailidis, P., Panagopoulos, P., Koutserimpas, C. Samonis, G. (2024). Current Therapeutic Approaches for Multidrug-Resistant and Extensively Drug-Resistant *Acinetobacter baumannii* Infections. Antibiotics. 13: 261.

Reza, A.A., Haque, M.A., Sarker, J., Nasrin, M.S., Rahman, M.M., Tareq, A.M., Khan, Z., Rashid, M., Sadik, M.G. Tsukahara, T. (2021). Antiproliferative and antioxidant potentials of bioactive edible vegetable fraction of *Achyranthes ferruginea* Roxb. in cancer cell line. Food Science and Nutrition. 9: 3777-3805.

Sadiq, A.Z.A., Ullah, F., Ayaz, S.A.M. (2014). Phytochemical and toxicological investigations of crude methanolic extracts, subsequent fractions and crude saponins of Isodon rugosus. Biological Research. 47: 1-6.

Siegel, R.L., Miller, K.D. Jemal, A. (2018). Cancer statistics, 2018. CA: A Cancer Journal for Clinicians. 68: 7-30.

Surh, Y.J. (2003). Cancer chemoprevention with dietary phytochemicals. Nature Reviews Cancer. 3(10): 768-780.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C. Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms. 9: 2041.

WHO (2023). https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.