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Preliminary Antibacterial Activity against Acne-Associated Bacteria, Cytotoxic Activity against HepG2 Liver Cancer Cells, and Phytochemical Profiling of Varthemia iphionoides Extracts from As-Salt, Jordan.

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ABSTRACT

Background: Varthemia iphionoides (V. iphionoides), locally known in Arabic as “Kuttelah”, is a wild plant grown in As-Salt Province. It is used among locals as a medical plant in the treatment of different diseases.

Aims: This study aimed to investigate effects of aqueous and 70% ethanolic extracts of V. iphionoides against acne-associated bacteria, as well as their cytotoxic effects against colorectal (Caco-2) and liver (HepG2) cancer cell lines, accompanied by phytochemical profiling. with the phytochemical analysis of the extracts.

Methods: The antibacterial activity against S. aureus, S. epidermidis, and C. acnes was evaluated using the broth microdilution method in 96-well plates and the agar well diffusion method. Then, the minimum inhibitory concentration (MIC) was determined. The cytotoxic effect was evaluated using MTT assay against the colorectal cells, Caco-2, and liver cancer cells, HepG2. The phytochemical analysis was done using LC-MC/MC.

Results: The aqueous extract showed the highest inhibition against C. acnes (24.6 ± 0.4 mm), and no activity against both S. aureus and S. epidermidis, while the ethanolic extract showed a moderate effect against S. aureus (16.0 ± 0.8 mm), S. epidermidis (13.3 ± 1.8 mm), and C. acnes (16.0 ± 0.0 mm). The aqueous extract showed MICs of 6.25 mg/ml C. acnes. The ethanolic extract showed MIC values of 12.5 mg/ml against S. aureus, 50 mg/ml against S. epidermidis, and 20.8 mg/ml against C. acnes. The results of MTT assays showed that only the ethanolic extract has a cytotoxic activity against HepG2 cells with IC_{50} value of 45.4 µg/mL, while none of the extracts showed cytotoxicity against Caco-2 cells. The ethanolic extract contained fatty acids palmitic and linoleic acids, triterpenoids (lupeol, betulinic acid), sesquiterpenes, and flavonoids, including quercetin, rutin, isoquercetin, and chlorogenic acid, while these compounds were absent in the aqueous extract.

Conclusions: The ethanolic V. iphionoides extract has the potential activity against acne-causing bacteria and cytotoxicity against liver cancer cells, without affecting colorectal cancer cells. However, these findings are still considered as preliminary and further in vivo experiments are needed to confirm the findings of this study.

Keywords: Acne, HepG2, phytochemical analysis, skin infection, Varthemia iphionoides.

Introduction

Local communities in Jordan strongly believe that wild plants play an important role in promoting human health. Traditional knowledge holds that medicinal plants are effective while causing fewer unwanted side effects compared with synthetic drugs [1]. In addition, Islamic teachings encourage the use of certain medicinal plants for the prevention and treatment of diseases [2].As a result, folk medicine and ethnopharmacological practices remain widely used in Jordan, particularly in rural and semi-urban areas.

As-Salt Province is located in the northwestern part of Jordan and borders the West Bank and Israel. The As-Salt region is characterized by mountainous terrain and a climate and landscape

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similar to those of Nablus [3]. This geographical setting distinguishes As-Salt from the southern and eastern regions of Jordan, which are predominantly desert. Consequently, the ecological system of As-Salt Province is relatively diverse and unique compared with other regions of the country. Local residents of As-Salt Province traditionally use a wild plant known locally as "Kuttelah" for the treatment of various diseases, including gastrointestinal disorders and cancer. The name "Kuttelah" literally translates to "the plant that kills diseases," indicating the strong belief in its therapeutic value. There is also a common local belief that donkeys in the region rarely become ill because they frequently consume this plant. Traditionally, the plant is boiled in water before being used for medicinal purposes, and some people also consume it fresh as part of salads during daily meals.

The scientific name of "Kuttelah" is Varthemia iphionoides (Asteraceae family), a wild aromatic plant characterized by its dark green color and strong, distinctive scent [4]. Previous studies have reported several biological activities for this plant. Previous studies have reported that V. iphionoides possesses antiplatelet activity [5]. Regarding its anticancer potential, the essential oil of V. iphionoides exhibits anti-breast cancer effects using both in vitro and in vivo models [6]. Other studies have also reported anticancer activity against pancreatic cancer cell lines [7].

In addition, antimicrobial effects of V. iphionoides against certain Gram-positive bacteria, including Staphylococcus aureus (S. aureus), have been documented [8]. The present study aims to extend the investigation of the chemotherapeutic potential of V. iphionoides by evaluating its antibacterial and anticancer activities against targets not examined in previous studies, using plant material collected from As-Salt Province. Specifically, this study assesses the activity of V. iphionoides against acne-associated bacteria, given the high prevalence of acne among Jordanians [9], as well as against colon and liver cancer cell lines, which represent major cancer types in Jordan [10] and have not been previously studied in relation to this plant.

Methods

Antibacterial Activity

Bacterial Materials

Three bacterial species associated with skin acne infections were evaluated in this study: *S. Aureus* with ATCC (9144), *S. Epidermidis* with ATCC (51625), and *Cutibacterium. Acnes (C. Acnes)* with ATCC (11827). The bacterial isolates were obtained from Al-Razi microbiology laboratory (Amman, Jordan) and stored under standard conditions until use.

Cell Culture

Human colorectal adenocarcinoma (Caco-2) and hepatocellular carcinoma (HepG2) cell lines were obtained from American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Plant Extracts

V. iphionoides was collected from Al-Subaihi region in As-Salt province (Google map: 32.147940, 35.702457) during October 2024. The plant was identified by an expert Professor of Botany in Jordan (Prof. Ahmad Al-Dararkah, Al-Yarmouk University). The aqueous and 70% ethanolic extracts of *V. iphionoides* were obtained by maceration. Briefly, 300g of leaves were macerated in 1 liter of 70% ethanol and other container contained 1 liter of distilled water for three days. The containers were amber and the temperature was around 20˚C. After that, the leaves were removed through Whatman No. 1 filter paper. The aqueous extract was obtained through lyophilization, while the ethanolic extract was obtained through the rotary evaporator at 40˚C for one day. Specific weights of the aqueous extract were dissolved directly in distilled water to prepare a 100 mg/ml solution. The ethanolic extract was dissolved in a mixture of 900 µl of 15% DMSO, 100 µl of Tween-20, and 5 ml of distilled water to prepare an 83.3 mg/ml solution for the antibacterial assays, while it was dissolved in 0.1% DMSO with sterile normal saline to prepare a stock solution of 100mg/ml for the anticancer assays.

Agar Well Diffusion and Broth Microdilution Methods

The antibacterial activity of plant extracts was evaluated using the agar diffusion method, as described previously [11, 12]. A standard bacterial suspension of each bacterial isolate (*S. aureus, S. epidermidis*, and *C. acnes*) equivalent to 0.5 McFarland standard (≈1.5×10^8 CFU/ml) was first prepared. 100 µL of each bacterial suspension was then homogeneously spread onto the surface of the appropriate culture medium; nutrient agar for *S. aureus* and *S. epidermidis* and brain heart infusion agar (BHI) for *C. acnes* under anaerobic conditions. Sterile cork borers with an 8 mm diameter were then used to create agar wells, and then each well was filled with 200uM/L of the extracts at different concentrations. A negative control was used which contained only the solvent (15% DMSO, 20% Tween, and 5 ml of distilled water). The plates were incubated at 37°C for 24 h (anaerobic conditions for *C. acnes*), and the diameter of the growth inhibition zones was measured using a ruler. All experiments were performed in triplicates.

Minimum Inhibitory Concentration (MIC)

The MIC value was determined using a 96-well plate assay following the method described by [13]. Briefly, for *S. aureus* and *S. epidermidis*, 100 µL of nutrient broth was added to each well, while Brain Heart Infusion (BHI) broth was used for *C. acnes*. Accordingly, 100 µl of the plant extract was added to the first well of each row separately (Aqueous extract 100 mg/ml; ethanolic extract 83.3 mg/ml), then a two-fold serial dilution was performed across wells 2 through 12 by transferring 100 µL from each well to the next (originally containing 100 µL of broth), mixing well at each step, and discarding 100 µL from the last well to maintain a volume of 200 µL/well before inoculation. This resulted in the following concentrations: From the aqueous solution (initial concentration: 100 mg/mL): Final concentrations in wells 1 to 12: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, 0.10, 0.05 mg/mL. From the ethanolic solution (initial concentration: 83.3 mg/mL): Final concentrations in wells 1 to 12: 83.3, 41.65, 20.86, 10.41, 5.21, 2.60, 1.30, 0.65, 0.33, 0.16, 0.08, 0.04 mg/mL. After completing the dilution, 100 µL of a standard bacterial suspension (1×10^6 CFU/mL) was added to each well, and the plates were then incubated at 37°C for 18 hours (with anaerobic conditions for *C. acnes*). The MIC value was recorded as the lowest concentration at which no visible bacterial

growth occurred. All measurements were performed in triplicate.

MTT Assay

Cytotoxicity of *V. iphionoides* aqueous and 70% ethanolic extracts were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay as previously described [14]. Briefly, Caco-2 and HepG2 cells in logarithmic growth phase were seeded into 96-well plates at a density of 1000 cells/well for Caco-2 and 80000 cells/well for HepG2 cells in 100 µL of the culture medium. Plates were incubated for 24 hours at 37°C in 5% CO^2. Following incubation, culture medium was aspirated and replaced with 100 µL of fresh medium containing increasing concentrations of *V. iphionoides* ethanolic extract (0.07–100 µg/mL). Control wells received medium containing 0.1% DMSO (vehicle control). Each concentration was tested in triplicate. After 48 hours of treatment, 10 µL of MTT solution (5 mg/mL in phosphate-buffered saline, pH 7.4) was added to each well, and plates were incubated for an additional 4 hours at 37°C. Subsequently, the supernatant was removed, and 100 µL of DMSO was added to each well to dissolve the insoluble purple formazan crystals. Plates were shaken gently for 10 minutes at room temperature, and absorbance was measured at 570 nm using Glomax plate reader (Promega, WI, USA). The cell viability was calculated as described previously [14]. Results were expressed as mean percentage ± standard deviation (SD). The percentage viability was plotted against log-transformed extract concentrations, and the half-maximal inhibitory concentration (IC^{50}) value was calculated as the concentration required to reduce cell viability by 50% compared to the control. Each treatment concentration was tested in three technical replicate wells within each experimental run. The experiment was repeated on two independent occasions.

Identification of Bioactive Compounds in the Extracts

The chemical compounds in both extracts were analyzed by Liquid Chromatography with tandem mass spectrometry SHIMADZU LC-MC/MC (Kyoto, Japan) done in Smart laboratory (Amman, Jordan), as described previously [15].

Statistical Analysis

Data were expressed as mean ± SD. Statistical comparisons between control and treated groups were performed using one-way analysis of variance (ANOVA) followed by *Tukey post-hoc* test. Differences were considered statistically significant at $p < 0.05$. All biological assays were done in triplicate (n=3) per plate and repeated in two independent experiments to confirm reproducibility.

Results

Agar Well Diffusion Assay

The antibacterial activity of the aqueous and ethanolic extracts of *V. iphionoides* against *S. aureus*, *S. epidermidis*, and *C. acnes* showed inhibition zones ranged between 12.0 ± 2.3 mm to 24.6 ± 0.4 mm (Table 1). The aqueous extract showed the highest inhibition against *C. acnes* (24.6 ± 0.4 mm), and no activity against both *S. aureus* and *S. epidermidis*, while the ethanolic extract showed an inhibition effect against *S. aureus* (16.0 ± 0.8 mm), *S. epidermidis* (13.3 ± 1.8 mm), and *C. acnes* (16.0 ± 0.0 mm), whereas the negative control doesn't show any activity.

MIC Assay

MIC values were determined for the aqueous and ethanolic extracts of *V. iphionoides* against the bacterial strains that demonstrate inhibitory activity in the agar well diffusion (Table 2). The MIC values ranged from 6.25 mg/ml to 50 mg/ml. The aqueous extract showed a MIC value of 6.25 mg/ml against *C. acnes*. The ethanolic extract showed MIC values of 12.5 mg/ml against *S. aureus*, 50 mg/ml against *S. epidermidis*, and 20.8 mg/ml against *C. acnes*. The vehicles did not show inhibition against the tested bacteria.

Cytotoxicity Assay

The results of MTT assays showed that the aqueous extract showed no cytotoxicity against either HepG2 or Caco-2 cell lines at tested concentrations (up to 100 µg/ml). In contract, the ethanolic extract has a cytotoxicity against HepG2 cells with an IC_{50} value of 45.4 µg/mL, while it did not show cyctotoxicity on Caco-2 cells, even at high concentrations (>100 µg/ml). Figure 1 illustrates the cytotoxicity of the ethanolic extract of *V. iphionoides* against HepG2 cells. The vehicle (0.1% DMSO did not show inhibition of the cell viability.

Phytochemical Analysis

The ethanolic extract of *V. iphionoides* was shown to contain 42 identified compounds, as shown in Table 3. The extract was rich in volatile monoterpenes and oxygenated monoterpenes, including p-cymene, limonene, α- phellandrene, pinene, camphor, thymol, 1,8-cineole, borneol, linalool, geraniol, and α-terpineol. Some sesquiterpenes and their derivatives were also detected, including caryophyllene, γ- irone, β- oplopenone, humulenol, ledol, γ -cadinol, and farnesol. In addition, the extract contained phenolic and organic acids including vanillic acid, caffeic acid, ferulic acid, citric acid, chlorogenic acid, palmitic acid, and linoleic acid. Additionally, the ethanolic extract consisted of bioactive flavonoids, such as apigenin, luteolin, quercetin, penduletin, isoquercetin, and rutin, alongside triterpenoids and sterols including β- stigmasterol, lupeol, and betulinic acid. The highest % of constitutes were of quercetin (6.2%) and farnesol (5.1%), while thymol (0.3%) and methyl eugenol (0.2%) showed the lowest %.

Table 1. The inhibition zone of *V. iphionoides against S. aureus, S. epidermidis,* and *C. acnes*; the diameter was reported in mm ± SD, n=3.

Bacterial strain	Aqueous extract (mm)	Ethanolic extract (mm)	Negative control
S. aureus	No activity	16 ± 0.8	–
S. epidermidis	No activity	13.3± 1.8	–
C. acnes	24.6 ± 0.4	16 ± 0	–

Table 2. The MIC values of *V. iphionoides* against *S. aureus, S. epidermidis*, and *C. acnes*.

Bacterial strain	Aqueous extract (mg/ml)	Ethanolic extract (mg/ml)
S. aureus	ND	12.5 mg/ml
S. epidermidis	ND	50 mg/ml
C. acnes	6.25 mg/ml	20.8mg/ml

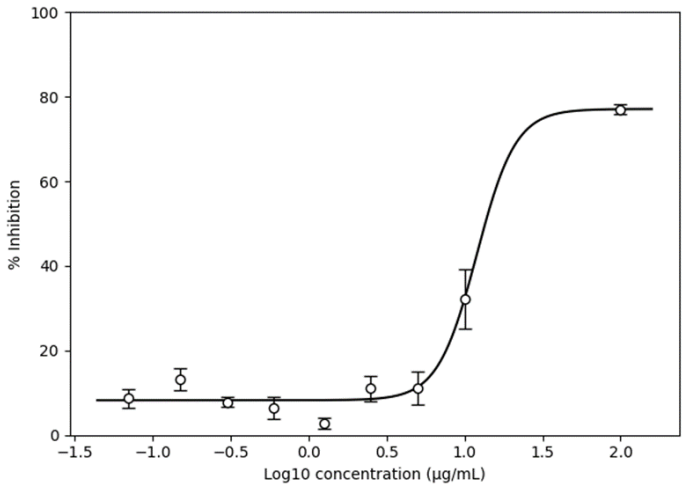

Figure 1. Percentage of the inhibition of cell viability of the ethanolic extract of *V.* iphionoides. Cells were treated with different concentrations of the extract (0.07–100 ug/mL) for 48 hours. Values are relative to the control (only the vehicle 0.1% DMSO). Data are presented as mean ± SD of three technical replicate wells from two independent experimental runs."*" indicates a statistical significance with $p < 0.05$ compared to control. Further information are found in Methods.

Table 3. Phytochemical analysis of the ethanolic extract of V. iphionoides.

Rank	Compound name	Molecular formula	Molecular weight (g/mol)	Relative %	RT (min)
1	Quercetin	$C_{15}H_{10}O_7$	302.23	6.2	39.0
2	Farnesol	$C_{15}H_{26}O$	222.37	5.1	34.0
3	Apigenin	$C_{15}H_{10}O_5$	270.24	4.6	36.0
4	Glutamine	$C_5H_{10}N_2O_3$	146.14	4.2	11.0
5	Palmitic acid	$C_{16}H_{32}O_2$	256.42	4.2	35.5
6	γ-Cadinol	$C_{15}H_{26}O$	222.37	4.1	32.2
7	Geraniol	$C_{10}H_{18}O$	154.25	4.1	19.5
8	Luteolin	$C_{15}H_{10}O_6$	286.24	4.0	38.5
9	Linoleic acid	$C_{18}H_{32}O_2$	280.40	3.8	38.0
10	β-Oplopenone	$C_{15}H_{24}O$	220.35	3.7	28.0
11	α-Phellandrene	$C_{10}H_{16}$	136.23	3.3	7.8
12	Caffeic acid	$C_9H_8O_4$	180.16	3.2	22.0
13	α-Fenchol	$C_{10}H_{18}O$	154.25	3.2	14.0
14	Bergamal	$C_9H_{16}O$	140.22	3.2	10.0
15	Rutin	$C_{27}H_{30}O_{16}$	610.50	3.1	48.5
16	Isoquercetin	$C_{21}H_{20}O_{12}$	464.40	3.0	46.2
17	Linalool	$C_{10}H_{18}O$	154.25	3.0	18.0
18	Penduletin	$C_{18}H_{16}O_7$	344.30	3.0	41.0
19	Borneol	$C_{10}H_{18}O$	154.25	2.7	17.4
20	Chlorogenic acid	$C_{16}H_{18}O_9$	354.31	2.2	42.0
21	Camphor	$C_{10}H_{16}O$	152.23	2.1	12.0
22	Vanillic acid	$C_8H_8O_4$	168.15	2.0	21.0
23	Limonene	$C_{10}H_{16}$	136.23	2.0	6.1
24	Bornyl acetate	$C_{12}H_{20}O_2$	196.29	2.0	25.0
25	Lupeol	$C_{30}H_{50}O$	426.70	2.0	43.8
26	Betulinic acid	$C_{30}H_{48}O_3$	456.70	1.9	45.0
27	β-Stigmasterol	$C_{29}H_{48}O$	412.70	1.7	43.0
28	Isophorone	$C_9H_{14}O$	138.21	1.5	9.5
29	Citric acid	$C_6H_8O_7$	192.12	1.4	23.0
30	1,8-Cineole	$C_{10}H_{18}O$	154.25	1.3	16.0
31	Geranyl acetate	$C_{12}H_{20}O_2$	196.29	1.2	25.9
32	trans-Carveol	$C_{10}H_{16}O$	152.23	1.0	13.1
33	γ-Irone	$C_{14}H_{22}O$	206.32	1.0	27.5
34	Pinene	$C_{10}H_{16}$	136.23	0.9	8.0
35	p-Cymene	$C_{10}H_{14}$	134.22	0.8	4.9
36	Ferulic acid	$C_{10}H_{10}O_4$	194.18	0.7	23.5
37	Humulenol	$C_{15}H_{24}O$	220.35	0.7	28.9
38	Caryophyllene	$C_{15}H_{24}$	204.35	0.5	27.1
39	α-Terpineol	$C_{10}H_{18}O$	154.25	0.5	16.9
40	Ledol	$C_{15}H_{26}O$	222.37	0.4	31.8
41	Thymol	$C_{10}H_{14}O$	150.22	0.3	11.5
42	Methyl eugenol	$C_{11}H_{14}O_2$	178.23	0.2	21.5

The aqueous extract of *V. iphionoides* contained different 38 identified compounds, as illustrated in Table 4. Monoterpene hydrocarbons and their derivatives, including p-cymene, limonene, α-phellandrene, pinene, isophorone, bergamal, thymol, camphor, trans-carveol, α-fenchol, 1,8-cineole, α-terpineol, borneol, linalool, and geraniol. The extract also contained organic and phenolic acids, such as vanillic acid, caffeic acid, ferulic acid, citric acid, and chlorogenic acid. In addition, sesquiterpenes and oxygenated sesquiterpenes including caryophyllene, γ-irone, β-oplopenone, humulenol, ledol, γ-cadinol, and farnesol. In addition, the aqueous extract was enriched with polyphenolic flavonoids, including apigenin, luteolin, quercetin, penduletin, isoquercetin, and rutin, as well as the phytosterol β-stigmasterol. The highest % of constitutes were of linalool (5.5%) and chlorogenic acid (5.5%), while α-Terpineol (0.2%) and methyl eugenol (0.1%) showed the lowest %.

Table 4. Phytochemical bioactive compounds identified in the aqeous extract of *V. iphionoides*

Rank	Compound name	Molecular formula	Molecular weight (g/mol)	Relative %	RT (min)
1	Linalool	$C_{10}H_{18}O$	154.25	5.5	18.0
2	Chlorogenic acid	$C_{16}H_{18}O_9$	354.31	5.5	42.0
3	Caffeic acid	$C_9H_8O_4$	180.16	5.0	22.0
4	Luteolin	$C_{15}H_{10}O_6$	286.24	4.2	38.5
5	Glutamine	$C_5H_{10}N_2O_3$	146.14	4.0	11.0
6	Geraniol	$C_{10}H_{18}O$	154.25	4.0	19.5
7	Farnesol	$C_{15}H_{26}O$	222.37	4.0	34.0
8	γ-Cadinol	$C_{15}H_{26}O$	222.37	3.7	32.2
9	Apigenin	$C_{15}H_{10}O_5$	270.24	3.6	36.0
10	Quercetin	$C_{15}H_{10}O_7$	302.23	3.5	39.0
11	Penduletin	$C_{18}H_{16}O_7$	344.30	3.5	41.0
12	Vanillic acid	$C_8H_8O_4$	168.15	3.3	21.0
13	β-Oplopenone	$C_{15}H_{24}O$	220.35	3.3	28.0
14	Limonene	$C_{10}H_{16}$	136.23	3.2	6.1
15	α-Fenchol	$C_{10}H_{18}O$	154.25	3.0	14.0
16	Bornyl acetate	$C_{12}H_{20}O_2$	196.29	2.6	25.0
17	β-Stigmasterol	$C_{29}H_{48}O$	412.70	2.6	43.0
18	α-Phellandrene	$C_{10}H_{16}$	136.23	2.5	7.8
19	Bergamal	$C_9H_{16}O$	140.22	2.5	10.0
20	Citric acid	$C_6H_8O_7$	192.12	2.2	23.0
21	trans-Carveol	$C_{10}H_{16}O$	152.23	2.1	13.1
22	Borneol	$C_{10}H_{18}O$	154.25	2.0	17.4
23	Geranyl acetate	$C_{12}H_{20}O_2$	196.29	2.0	25.9
24	Isoquercetin	$C_{21}H_{20}O_{12}$	464.40	1.9	46.2
25	Camphor	$C_{10}H_{16}O$	152.23	1.6	12.0
26	γ-Irone	$C_{14}H_{22}O$	206.32	1.5	27.5
27	Rutin	$C_{27}H_{30}O_{16}$	610.50	1.3	48.5
28	p-Cymene	$C_{10}H_{14}$	134.22	1.2	4.9
29	Isophorone	$C_9H_{14}O$	138.21	1.2	9.5
30	Humulenol	$C_{15}H_{24}O$	220.35	1.2	28.9
31	Pinene	$C_{10}H_{16}$	136.23	1.1	8.0
32	1,8-Cineole	$C_{10}H_{18}O$	154.25	1.0	16.0
33	Caryophyllene	$C_{15}H_{24}$	204.35	0.7	27.1
34	Ferulic acid	$C_{10}H_{10}O_4$	194.18	0.5	23.5
35	Ledol	$C_{15}H_{26}O$	222.37	0.5	31.8
36	Thymol	$C_{10}H_{14}O$	150.22	0.6	11.5
37	α-Terpineol	$C_{10}H_{18}O$	154.25	0.2	16.9
38	Methyl eugenol	$C_{11}H_{14}O_2$	178.23	0.1	21.5

Discussion

V. iphionoides is widely used in As-Salt region by local communities. However, scientific evidence on its beneficial effects still under investigations. In this study, we continued the screening of the potential chemotherapeutic activity of the ethanolic and water extracts of of *V. iphionoides* using *in vitro* methods and analyzed the phytochemical bioactive compounds using high throughout LC-MC/MC method. We found, in this study, that the ethanolic extract of *V. iphionoides* has a high potential to be further investigated in future as anti-acne and in the prevention and treatment of hepatic cancer. Further studies using *in vivo* animal and human models are needed to confirm these findings.

In the present study, the aqueous extract of *V. iphionoides* showed little to no antibacterial activity against *S. aureus* and *S. epidermidis*. In addition, the aqueous extract did not exhibit any *in vitro* cytotoxic effects against either HepG2 or Caco-2 cancer cell lines. Its antibacterial activity was limited to *C. acnes* only. In contrast, the ethanolic extract demonstrated both broader antibacterial activity and anticancer effects. It was active against all tested acne-associated bacterial strains and exhibited cytotoxicity toward HepG2 liver cancer cells, while no cytotoxic effect was observed against Caco-2 cells. Phytochemical analysis revealed that the ethanolic extract possessed a more diverse chemical composition, with 42 identified compounds compared with 38 compounds in the aqueous extract, and generally higher relative levels of lipophilic constituents. Notably, several non-polar bioactive compounds, including palmitic acid, linoleic acid, lupeol, betulinic acid, and other triterpenoids, were detected exclusively in the ethanolic extract. This extract also contained higher proportions of certain sesquiterpenes and high-molecular-weight flavonoids, such as quercetin, rutin, isoquercetin, and chlorogenic acid. Accordingly, these findings suggest that the lipophilic compounds enriched in the ethanolic extract may play a key role in mediating both the antibacterial activity and the observed cytotoxic effect against liver cancer cells. Although traditional practices in As-Salt Province commonly involve the use of aqueous preparations of *V. iphionoides* for various therapeutic purposes, our results indicate that the ethanolic extract exhibits superior biological activity, particularly as a potential anti-acne and anti-liver cancer agent.

Phytochemical profiling revealed that the ethanolic extract is richer in lipophilic constituents, which are more capable of penetrating the thick peptidoglycan layer characteristic of Gram-positive bacterial cell walls [16]. This extract contained fatty acids (palmitic and linoleic acids), triterpenoids (including lupeol, betulinic acid, and related compounds), sesquiterpenes, and high-molecular-weight flavonoids such as quercetin, rutin, isoquercetin, and chlorogenic acid. These compounds are widely recognized for their antibacterial properties, particularly against Gram-positive bacteria [17, 18]. Fatty acids can compromise membrane integrity by increasing membrane fluidity and permeability, ultimately leading to leakage of intracellular components [19]. Triterpenoids are known to integrate into lipid bilayers, disrupt membrane structure, inhibit essential bacterial enzymes, and induce oxidative stress [20]. Additionally, flavonoids such as quercetin and rutin can inhibit nucleic acid synthesis, interfere with bacterial energy metabolism, and destabilize the cell wall [21]. The coexistence of these bioactive constituents likely produces synergistic effects, which can result in a stronger and broader antibacterial activity observed with the ethanolic extract of *V. iphionoides*.

The aqueous extract of of *V. iphionoides* is dominated by more polar compounds and lacks many of the lipophilic antibacterial agents present in the ethanolic extract. This compositional difference might reduce effectiveness of the aqueous extract against *S. aureus* and *S. epidermidis*, whose membranes are particularly vulnerable to lipophilic molecules. However, the aqueous extract retained activity against *C. acnes*, which may be due to the unique biological characteristics of this bacterium. *C. acnes* is an anaerobic, lipid-dependent organism that inhabits sebaceous environments and is especially sensitive to polar phenolic compounds and organic acids [22].

The antibacterial effect of the aqueous extract against *C. acnes* might be explained by its higher content of polar phenolic acids (caffeic and chlorogenic acids), flavonoids (luteolin and apigenin), organic acids such as citric acid, and oxygenated monoterpenes (including thymol, camphor, 1,8-cineole, and linalool). These compounds are known to disrupt cellular redox balance, inhibit key metabolic enzymes, alter membrane permeability, and reduce intracellular pH-mechanisms that are particularly detrimental to *C. acnes* due to its anaerobic metabolism [23, 24].

The MTT assay results showed that only the ethanolic extract of *V. iphionoides* exhibited a concentration-dependent cytotoxic effect on HepG2 cells, with an IC_{50}value of 45.4 μg/mL. According to established criteria for plant extract cytotoxicity [25], an IC_{50} between 20–100 μg/mL is considered as a moderately active. Therefore, the ethanolic extract of *V. iphionoides* showed a moderate cytotoxicity and a potentiality against liver cancer cells.

However, the same extract did not exhibit cytotoxic effects against colorectal Caco-2 cells in this study. This suggests that the cytotoxic activity of *V. iphionoides* may be cell type–specific, due to the differences in biochemical pathways between cancers [26]. HepG2 cells retain active hepatic lipid-handling and mitochondrial pathways, including fatty acid uptake, β-oxidation, and mitochondrial apoptosis signaling [27]. Several compounds identified only in the ethanolic extract, such as palmitic acid, linoleic acid, lupeol, betulinic acid, and other triterpenoids, are known to induce lipotoxicity, trigger mitochondrial membrane depolarization, activate caspase-dependent apoptosis, and disrupt cholesterol and membrane lipid homeostasis [28, 29]. However, these are still hypotheses regarding the cytotoxic effects of *V. iphionoides* and further studies are needed to elucidate its mechanism of the anticancer activity against HepG2 cells.

In contrast, Caco-2 cells are characterized by high expression of efflux transporters, including P-gps, which actively pump out lipophilic terpenoids [30]. As a result, Caco-2 cells are more resistant to the cytotoxic effects of these compounds, which might explain the lack of activity in this cell line.

The essential oils of V. iphionoides showed a cytotoxicity against colorectal cell lines, including Caco-2 [31]. This discrepancy in the results regarding the cytotoxicity of V. iphionoides against Caco-2 cells might be due to different methods of extractions, where we used in this study the ethanolic extract, while the study of

Alqaraleh et al. used the concentrated essential oils [31]. These findings suggest that different extraction methods for *V. iphionoides* yield distinct phytochemical profiles, which in turn lead to variations in biological activity against cancer cells.

Both studied cell lines in this study are cancer-derived and non-cancerous cells were not evaluated. Therefore, these findings do not establish cancer selectivity. Further studies including non-cancerous cell are required to determine the selectivity of the extract against hepatic cancer cells.

Although this study showed preliminary results into the antibacterial and anticancer activities of *V. iphionoides*, some limitations should be acknowledged. First, standard reference controls, including established antibiotics for the antibacterial assays and a reference cytotoxic agent for the MTT assay, were not included. Consequently, the observed effects cannot be compared directly with those of established treatments, and the relative potency of the extracts cannot be determined. Second, while detailed LC-MC/MC profiling identified key candidate phytochemicals, individual isolated compounds were not separately evaluated to experimentally confirm their specific mechanistic contributions or potential synergistic interactions responsible for the observed bioactivities. Finally, due to budget and resource constraints, cytotoxicity was not evaluated against non-cancerous/normal cell lines, such as normal human hepatocytes or skin fibroblasts. Future studies will focus on isolating major bioactive constituents and including standard reference controls to fully validate the clinical potential of *V. iphionoides*.

Conclusion

In conclusion, the ethanolic extract of *V. iphionoides* demonstrates preliminary anti-acne and anti-hepatocellular carcinoma activities *in vitro*, without affecting colorectal cancer cells. However, further studies including positive controls of antibacterial and anticancer, non-cancerous cell lines, and experiments designed to validate the mechanisms underlying the observed effects. of *V. iphionoides*.

Conflict of Interest

The authors declare that there is no conflict of interest

Data Availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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