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Research paper

Antioxidant, antimicrobial and cytotoxic properties of four different extracts derived from the aerial parts of *Chiliadenus iphinoides*

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ABSTRACT

Introduction: *Chiliadenus iphinoides* is a Mediterranean basin plant and utilized in traditional medicine in Palestine. Thus, the aim was to investigate, for the first time, antioxidant, antimicrobial and cytotoxic properties of *C. iphinoides* gathered from Jenin-Palestine.

Methods: The DPPH technique was used to measure antioxidant activity. Antimicrobial activity was tested employing broth microdilution and agar-well diffusion methods. Anti-proliferative and apoptotic activities were evaluated utilizing colorimetric methods.

Results: Aqueous, methanol, acetone and hexane extracts and trolox exhibited antioxidant activities with IC50 values of 5.2, 7.5, 4.2, 218 and 0.8 μg/ml, respectively. Acetone extracts had the most potent and broad spectrum antimicrobial activity followed by hexane and then methanol extracts, while aqueous extracts had no effect except against *S. sonnei.* Both hexane and acetone extracts had strong anti-proliferative effects followed by methanol extracts. Caspase-3 activity was induced by hexane extract 2 folds, similar to doxorubicin effect, a chemotherapeutic medicine.

Conclusion: In the current study we demonstrated that acetone and hexane extracts possessed a strong anti-proliferative property compared to methanol extract. Thiswas mediated via apoptosis; suggesting that they represent an attractive source of bioactive anticancer molecules. Acetone, hexane and methanol extracts possess broad spectrum antimicrobial effects. Acetone, aqueous and methanol extracts have strong antioxidant effects. Interestingly, aqueous extract is a potential safe source of antioxidant agents and natural preservatives instead of chemical preservatives in pharmaceutical and food products. However, it is necessary to validate these findings *in vivo* in the future.

1. Introduction

Plants and their natural products represent an attractive and valuable source of biologically active compounds [1]. They are composed of multiple compounds such as flavonoids, phenols, saponins glycosides, and cyanogenic compounds and therefore a plant may possess multiple biological activities such as antioxidant, antibacterial and cytotoxic activities, which can treat, cure and/or prevent various diseases. So, it is no surprise that 50% of all drugs in clinical use are derived from plants. The Eastern part of the Mediterranean, including Palestine, is a rich region with various species of plants; approximately 3600 plant species grow in this region. Only a small extract of these plants, around 450–550 species, have been used in herbal medicine [2]. Consequently, it is essential to screen plants in this region used in traditional medicine based on evidence for their potential applications in medicine [3].

Chiliadenus iphionoides (Boiss. & Blanche) Brullo is a member of the Asteraceae family. Its synonym name is *Varthemia iphionoides* Boiss. & Blanche, which is locally known as "أشبتيله". *C. iphinoides* is a small shrubby perennial plant that grows in Mediterranean basin region (Palestine, Jordan, Syria and Lebanon) [4,5]. It grows in semidry land and rocky places [6]. It is a perennial small shrup with a woody base, many-branches, small leaves, aromatic, hairy and sticky stems and has tubular yellow flowers that flowering from September to December [7]. It is commonly used in traditional medicine in the Middle East, including Palestine, as infusion or decoction of aerial parts to treat cold, fever, influenza, stomachache, depression and nervousness, kidney stones, eye

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infections, also it has been used as antispasmodic [4]. There is a little scientific evidence that supports its traditional use. Beside this, it is well known that plant species vary from region to region and so they possess different biological properties according to their region's environment [8]. To our knowledge, this is the first paper in which we investigate *C. iphinoides* gathered from Palestine for its biological activities, i.e. antioxidant, antimicrobial and cytotoxic activities.

2. Materials and methods

2.1. Collection and preparation of C. iphionoides aerial parts

Aerial parts of *C. iphionodes* were collected in October 2016 from the mountains of Kafr-Raie located in Jenin-Palestine. The plant has been botanically identified by pharmacognosist Dr. Nidal Jaradat from the Pharmacy Department at An-Najah National University. A voucher specimen (Pharm-PCT-602) has been retained in the herbarium of the Laboratory of Pharmacognosy. The plant was washed well several times with distilled water and then dried in a well-shaded place at room temperature for 2 weeks. The dried plant was kept in special containers for future use.

2.2. Extraction procedure

The extraction process was processed using distilled water, as an aqueous solvent, and three different organic-solvents, i.e. methanol, acetone and hexane, in order to have a wide-range of polarity of organic extracts [9]. *C. iphinoides* aerial parts were grounded into a fine powder. Approximately, 50 g of the powder were soaked in a half liter of each of the aqueous and organic solvents and kept for about 72 h at a shaker incubator (100 RPM at room temperature). Then, each of the plant extracts were filtered by using Buchner funnel and filter papers. The resulting three organic extracting-solvents were vaporized by a rotary evaporator under high pressure at maximal temperature of 35 ºC. On the other hand, the resulting aqueous solvent was vaporized by using freezing drier machine. The resulting aqueous and organic extracts were stored for further use at 4ºC [9,10].

2.3. In vitro evaluation of anti-oxidant activity via DPPH radical method

The anti-oxidant activity of each of the obtained aqueous and organic extracts were evaluated using the DPPH radical method [11]. Stock solutions of the four extracts and Trolox were prepared in methanol (1mg/ml). Then, the following concentrations were prepared 100, 80, 50, 40, 30, 20, 10, 7, 5, 3, 2, 1µg/ml. Working-solutions were prepared by mixing 1ml of each of diluted solution with 1ml of methanol and 1 ml of a freshly-prepared DPPH solution and incubated in a dark room at room temperature for 30 min. Optical density of the reaction mixtures (in triplicates) were measured at 517 nm spectrophotometrically. Absorbance of the DPPH radical without antioxidant, i.e. blank was also measured. DPPH radical inhibition was calculated as following (1):

DPPH radial scavenging activity = ((A0 - A1) / A0)) X 100%, where, A0 and A1 are the optical densities of Trolox standard and the working solution at 30 min, respectively.

The antioxidant activity of the tested material was expressed as IC_{50} (µg/mL) that represents the concentration of that material which causes a 50% decrease in the optical density at the wave length of 517 nm. This was inversely-proportional between the value of IC50 of the material and its antioxidant activity.

2.4. Antimicrobial activity

2.4.1. Microbial isolates

Antibacterial properties of the four extracts were tested against 6 types of reference bacterial strains purchased from the American Type Culture Collection (ATCC), i.e. *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Shegilla sonnie* (ATCC 25931), *Enterococcus faecium* (ATCC 700221), *Staphylococcus aureus* (ATCC 25923) and *Methicillin Resistance Staphylococcus aureus* (*MRSA*) clinical isolate. Antifungal activity was determined against *Candida albicans* (ATCC 90028) and *Epidermatophyton floccosum* (ATCC 52066).

2.4.2. Preparation of plants extracts to test the antimicrobial activities

25 mg/ml of the hydrophilic and hydrophobic extracts were dissolved in sterile distilled water and Dimethyl sulfoxide (DMSO), respectively. These solutions were sterilized using 0.45 µm filters.

2.4.3. Inoculums preparations

Inoculums of fresh bacterial isolates were suspended in sterile normal saline until turbidity was equivalent to 0.5 McFarland standard (1.5×10^8 CFU/ml), then the suspension was diluted and the final concentration was 5×10^5 CFU/ml [12].

2.4.4. Broth micro-dilution method

Solutions of plant's extracts were two-fold serially diluted with Mueller-Hinton II Broth 11 times. Mueller-Hinton II broth free of plant's extracts was used as a positive control of microbial growth. Wells were inoculated with or without various types of bacteria in duplicates. Inoculated plates were incubated at 35 °C for 24 h. The lowest concentration of plant extracts that did not allow any visible growth of microbe in the test broth was considered the minimal inhibitory concentration (MIC) [13].

2.4.5. Agar-well diffusion assay

1.5×10^8 CFU/ml of the bacterium was subcultured on the Mueller-Hinton agar medium. Wells (each 6 mm in diameter) were made in agar plates. 80 µl of each plant extracts was added to each well. The inoculated dishes were incubated at 35 °C for 24 h, then the diameter of the bacterial inhibition zone was measured [14]. Each plant extract was examined in a duplicate.

2.4.6. Cell culture, proliferation and caspase-3 activity assays

HeLa cervical adenocarcinoma cell line was cultured in RPMI-1640 media, which was supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin antibiotics and 1% l-glutamine. Cells were grown in a humidified atmosphere with 5% CO_2 at 37°C. Cells were treated as described earlier [15]. Cells were treated with 2.5, 1.25, 0.625, 0.3125 and 0.0 mg/ml of each extract and further incubated for 4, 24 and 48h. Organic extracts (methanol, acetone and hexane) were dissolved in DMSO, while aqueous extract was dissolved in RPMI-1640 media. A concentration of 10 µM of doxorubicin was used as a positive control for anti-proliferative activity. Anti-proliferative effect of the plant extracts was assessed by Cell Tilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the manufacturer's instructions (Promega Corporation, Madison, WI). Caspases-3 activity of hexane extract and doxorubicin (positive control) treated cells was measured according to the manufacturer guidelines (biovision, MA, USA).

2.5. Statistical analysis

GraphPad Prism software version 6.01 was used to perform the statistical analysis. Comparison between 2 groups was analyzed via t-test. IC_{50} was determined employing nonlinear regression test. Comparison between multiple groups belong to two factor was analyzed via two-way ANOVA followed by Bonferonni's post hoc test. Results were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Antioxidant activity

DPPH radical scavenging activities induced by various test

concentrations of aqueous, methanol, acetone, and hexane extracts derived from the aerial parts of *C. iphinoides* are demonstrated in Fig. 1. There was a significant dose–dependent increase in the percentage of antioxidant activities for all tested concentrations of aqueous, methanol, acetone extracts and hexane, however, with a different extent. IC50 values (Table 1) of aqueous, methanol, acetone, and hexane extracts were 5.2, 7.5, 4.2 and 218 µg/ml, respectively, while the IC50 value of trolox, the standard material, was 0.7 µg/ml.

3.2. Antimicrobial activity

As demonstrated in Table 2, aqueous extract of *C. iphinoides* had no antimicrobial activity against the tested microbes except against *S. sonnie* with MIC values of 3 mg/ml. Methanol extract had antibacterial activity against *S. aureus* with MIC value 1.6 mg/ml and had a similar effect on *MRSA, E. faecium, E. coli* and *P. aeruginosa* with MIC values of 6.3 mg/ml, while it had no effect on *S. sonnie*. In addition, methanol extract possessed antifungal activity against *C. albicans* with MIC value of 6.3 mg/ml. Acetone extract had potent antimicrobial activity against of *S. aureus, MRSA, E. faecium, E. coli, S. sonnie, P. aeruginosa* and *C. albicans* with MIC values 0.4, 6.3, 0.4, 0.8, 3, 3, 1.6 mg/ml, respectively. Hexane extract had antimicrobial activity against *S. aureus, MRSA, E. faecium, E. coli, S. sonnie, P. aeruginosa* and *C. albicans* with MIC values of 1.6, 6.3, 1.6, 12.5, 3, 3 and 1.6 mg/ml, respectively. None of the tested extracts had any effect against *E. floccosum*. These results were validated using the agar diffusion method as depicted in Table 3. Aqueous extract of *C. iphinoides* did not produce inhibition zones against any type of tested bacteria except for *S. sonnie* with inhibition zone diameter 20 mm. Methanol extract produced inhibition zone diameter of 14, 11, 15,10 and 10 mm against *S. aureus, MRSA, E. faecium, E. coli*, and *P. aeruginosa*, respectively. Acetone extract produced inhibition zone diameter of 11, 9, 13, 4, 4 and 3 mm against *S. aureus, MRSA, E. faecium, E. coli, S. sonnie* and *P. aeruginosa*, respectively. Hexane extract produced inhibition zone diameter of 11, 7, 3, 1, 6 and 1 mm against *S. aureus, MRSA, E. faecium, E. coli, S. sonnie* and *P. aeruginosa*, respectively.

3.3. Anti-proliferative activity

HeLa cervical adenocarcinoma cells were treated with 0.0, 0.3125, 0.625, 1.25, and 2.5 mg/ml of aqueous, methanol, acetone and hexane extracts derived from the aerial parts of *C. iphionoides*. Three time points were investigated, i.e. 4, 24 and 48 h. Exposure to aqueous extract for 4 and 24 h had no effect on cells' cytotoxicity, while treatment with 2.5 and 1.25 mg/ml for 48 h decreased cell viability significantly (p-value <0.05) to 13% and 17%, respectively (Fig. 2D). Treatment with methanol extract (all tested concentrations) for 4 h inhibited cell viability significantly (p-value <0.01) to approximately 50%, while treatment for 24 h decreased cell viability in a dose dependent manner to 10% (2.5 mg/ml, p-value <0.0001), 41% (1.25 mg/ml p-value <0.0001) and 66% (0.625 mg/ml, p-value <0.05) (Fig. 2C). As depicted in Fig. 2A and B, 4 h treatment with both hexane and acetone extracts reduced the cell viability significantly in a dose dependent manner (from 10 to 50%), while treatment for 24 h with the highest concentrations (2.5–0.625 mg/ml) inhibited cell viability to approximately 10%, while 0.3215 inhibited the cell viability to 70%. Treatment for 48 h by all tested concentrations of both acetone and hexane extracts decreased significantly (p-value <0.0001) the viability of cells to approximately 10% (Fig. 2A and B). Treatment with doxorubicin (positive control) decreased cell viability to approximately 50% at all time points.

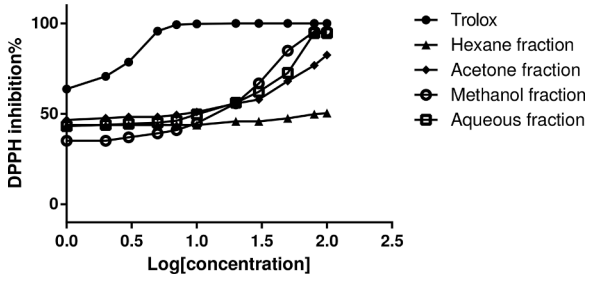

Fig. 1. Dose-response inhibition of radical scavenging activity. 100, 80, 50, 40, 30, 20, 10, 7, 5, 3, 2, 1µg/ml of aqueous, methanol, acetone and hexane extracts derived from *C. iphinoides* were tested for radical scavenging activity employing DPPH method. Results were depicted as percentage of inhibition. Trolox was used as the reference standard.

Table 1
IC_{50} values of trolox standard reference and fractions derived from *C. iphinoides.*

Fraction / reference standard	IC_{50} µg/ml (mean ± SEM)	95% CI
Aqueous	5.2 ± 1.2	3.6 to 7.4
Methanol	7.5 ± 1.1	5.9 to 9.6
Acetone	4.2 ± 1.2	3 to 5.9
Hexane	218 ± 1.7	70.3 to 676.3
Trolox (reference standard)	0.73 ± 1.1	0.6 to 0.89

SEM: standard error of the mean, CI: confidence interval.

Table 2
Antimicrobial activity of fractions derived from *C. iphinoides* determined by minimum inhibitory concentration method (mg/ml).

Bacterial species	MIC (mg/ml)			
	Aqueous	Methanol	Acetone	Hexane
Staphylococcus aureus	NA	1.6	0.4	1.6
MRSA	NA	6.3	6.3	6.3
Enterococcus faecium	NA	6.3	0.4	1.6
Escherichia coli	NA	6.3	0.8	12.5
Shigella son25e	3	NA	3	3
Pseudomonas aeruginosa	NA	6.3	3	3
Candida albicans	NA	6.3	1.6	1.6
Epidermatophyton floccosum	NA	NA	NA	NA

NA: no activity

Table 3
Antimicrobial activity of fractions derived from *C. iphinoides* determined by agar diffusion assay (diameter-mm).

Bacterial species	Inhibition zone mm			
	Aqueous (WC)	Methanol (MC)	Acetone (AC)	Hexane (HC)
Staphylococcus aureus	0	14	11	11
MRSA	0	11	9	7
Enterococcus faecium	0	15	13	3
Escherichia coli	0	10	4	1
Shigella son25e	20	0	4	6
Pseudomonas aeruginosa	0	10	3	1

mm: millimeter.

3.4. Caspase-3 activity

As hexane and acetone extracts of *C. iphionoides* possessed the strongest anti-proliferative effect, caspase activity was evaluated for hexane and doxorubicin was used as a positive control at a concentration of 10 µM. As demonstrated in Fig. 3A and B, treatment with doxorubicin (dox) and hexane extract increased the activity of caspase-3 significantly ($p < 0.05$) by 1.8 and 2.4 folds, respectively.

4. Discussion

Plants were the source of most medicinal preparations throughout the human history and have continued to be a major source of natural

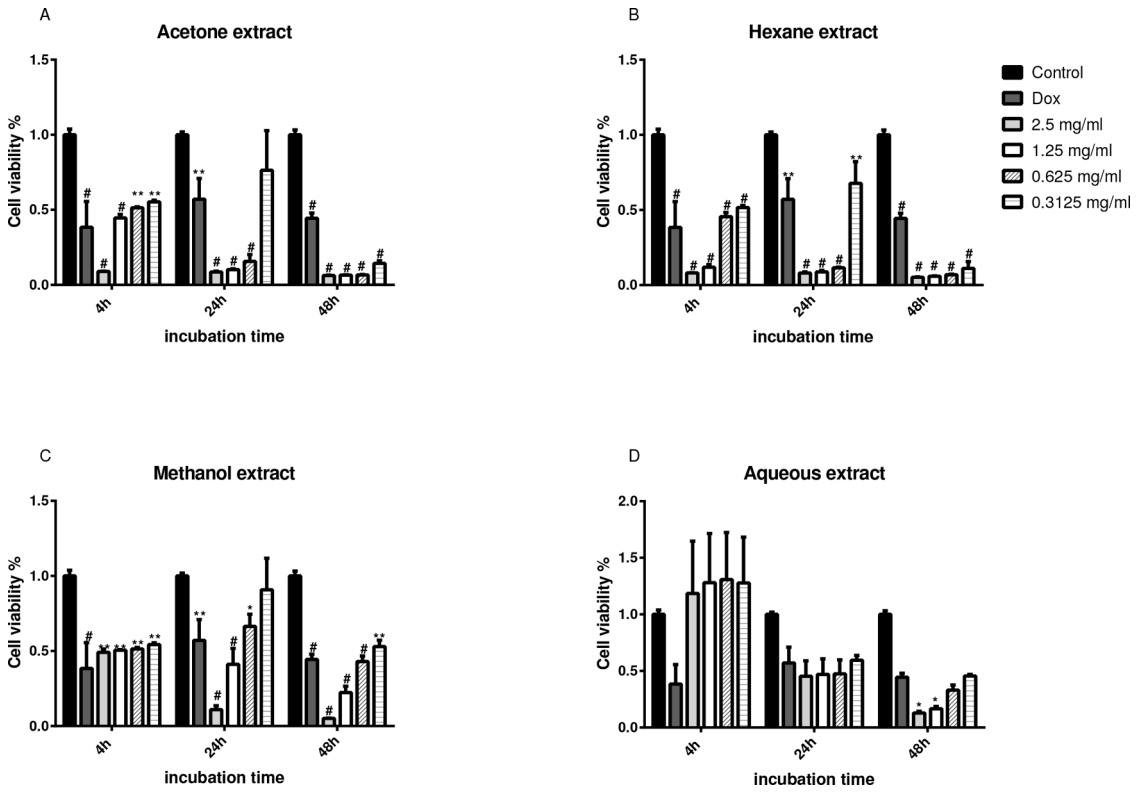

Fig. 2. Anti-proliferative effect of (A) acetone, (B) hexane, (C) methanol, (D) and aqueous extracts obtained from *C. iphinoides*. Hela cells were treated with 2.5, 1.25, .625 and .3125 mg/ml of acetone, hexane, methanol and aqueous extracts obtained from *C. iphinoides* and incubated for 4, 24 and 48 h. Proliferation was determined by MTS assay. Results were depicted as relative quantities (RQs) compared to the control (C; with only media for aqueous extract and DMSO and media for organic extracts). *#P<0.0001*, ***P<0.01* and **P<0.05*. Error bars represent SD.

products that enter clinical trials, especially as anticancer and antimicrobial agents. This is clearly shown by the fact that 75% of medicines that were approved by the US Food and Drug Administration (FDA) between 1981 and 2014 were not synthetic, 49% were natural products or direct derivatives of them and 1% was defined mixture of plants [16,17]. *C. iphinoides* is a plant grown in the Middle East and it has been used in the traditional medicine in Palestine. However, there is little evidence that support this use and it has never been investigated in Palestine. It is well recognized that chemical composition of a plant is influenced by a variety of elements, including geographic location, harvest time, environmental conditions, investigated parts of plants and experimental setup [18,19]. This also is demonstrated by the fact that different research studies found varying compositions of *C. iphinoides* as reviewed extensively by Abdelhalim et al. [20]. Therefore, in the current study, we have screened the aerial parts of *C. iphinoides* collected from Jenin-Palestine for their antioxidant, antimicrobial and anticancer activities.

Although antibiotics usage has decreased the spread and severity of many infectious diseases; infectious diseases are still the top and second leading causes of death in low- and high-income countries [21]. The uncontrolled and widespread use of antibiotics has lead to antibiotic resistance. Thus, it is not surprising that the number of people who will die due to antibiotic resistance would increase from 700,000 to 10 million per year globally by 2050 according to the World Health Organization (WHO) estimation [22]. Examples of known bacteria that develop antibiotic resistance are *Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella species* [23]. Therefore, we have investigated the antibacterial activities of extracts derived from *C. iphinoides* aerial parts as a natural alternative of synthetic antibiotics and we have found that acetone extract had the most potent and broad-spectrum antibacterial activity followed by hexane and then methanol extracts, while aqueous extract had no effect at all tested bacteria except against *S. sonnei.* The observed limited aqueous effect is in agreement with an earlier study on *C. iphinoides* collected from Jordan [24], except for *S. sonnei* gram negative bacterial species, which we have shown to possess an antimicrobial effect with MIC value of 3 mg/ml. This represents a promising application as natural preservative instead of chemical preservatives in pharmaceutical and food products. On the other hand, methanolic extract was tested in one study [14] and our study has demonstrated a stronger anti-*E. coli* effect (10 mm vs 1.68 mm) and a weaker anti-*MRSA* (11 vs 21). In contrast to our study, the methanolic extract was derived from the leaves that were collected from Jordan and the tested concentration was higher than our tested concentration. Similar low sensitivity against *E. coli* to *C. iphinoides* was also reported by Masadeh et al. and Abu-Hijleh et al. [25,26]; however, these were collected from Jordan and extracted via ethanol rather than methanol. Acetone extract, followed by hexane extract, has appeared to possess the strongest antibacterial effect with very low MIC values against all types of tested bacteria species especially against *E. Coli* with a MIC value of 0.8 mg/ml. This is even stronger than common reference antibiotics such as cefuroxime, amoxicillin and ampicillin that have a MIC value of 4 mg/ml against *E. coli* [27]. This is in contradictory to Al-Dabbas et al. [24] findings; since, hexane and ethanolic extracts from the aerial parts had no antimicrobial effects. This could be due to different setup of the experiments and different source of *C. Iphionoides,* as it was collected from Jordan. The observed strong antimicrobial activities of *C. iphionoides* aerial parts in our study support their traditional use in Palestine against infectious diseases [28]. With respect to antifungal effect, acetone and hexane extracts had a strong effect against *C. albicans* (1.6 mg/ml) followed by methanolic extract (6.3 mg/ml). Acetone and hexane extracts observed effects are stronger than fluconazole, a common reference antibiotic, which has a MIC of 4.196 mg/m. For many years ago, amphotericin B was the only available and potent treatment for fungal infections. Nowadays, the most widely

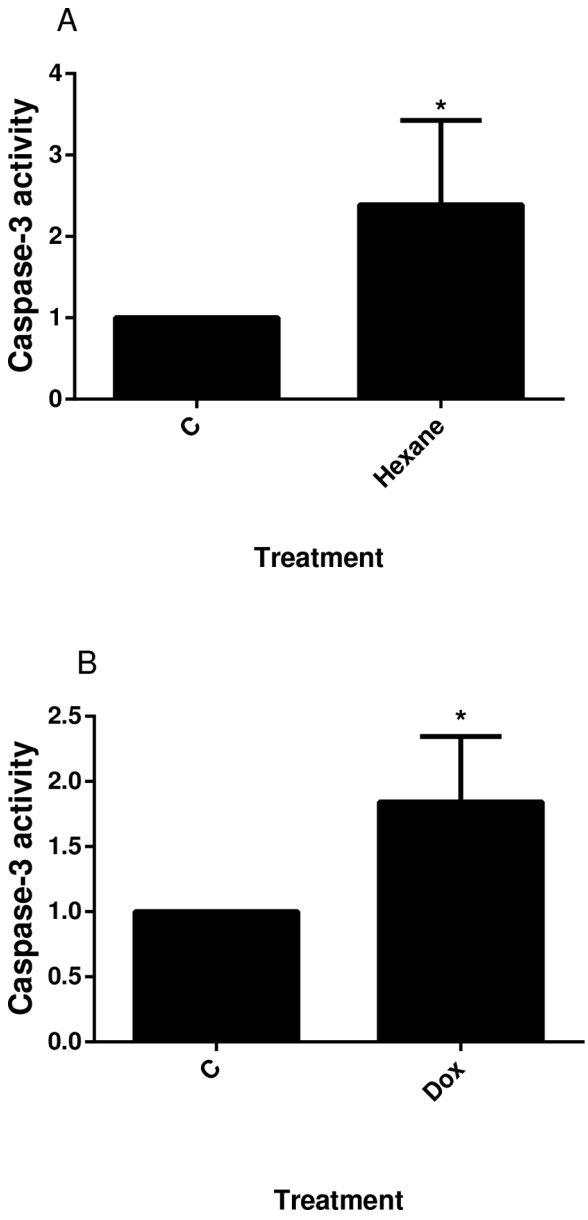

Fig. 3. Effect of hexane extract obtained from *C. iphinoides* on Caspase-3 activity. Hela cells were treated with 0.6215 mg/ml of acetone extract derived from *C. iphinoides* for 48 h. Caspase-3 activity was determined using caspase-3 /CPP32 Colorimetric Protease Assay kit. Results were depicted as relative quantities (RQs) compared to the control (with media and DMSO; C). $*P < 0.05$. Error bars represent SD. Dox: doxorubicin

used antifungal agents are azole derivatives [29]. All used available antifungal drugs till today are not ideal in efficiency and safety [30]. Therefore, *C. iphinoides* represents an alternative source of natural products against *C. albicans*. In summary, although all earlier studies, used different extracts, different extraction methods, tested different microbes, used different antimicrobial assays and used different parts and regions of *C. iphionoides,* however, all support the notion that all extracts, except water, of *C. iphionoides* possess a potent antimicrobial effect.

An imbalance between oxidants and antioxidants in favor of oxidants is termed “oxidative stress. An example of oxidants is reactive oxygen species (ROS) that are generated in body and cause irreversible damage to the cell components such as DNA, lipids and proteins that lead to tissue injury [31] and consequently to uncurable diseases such as Alzheimer disease, coronary heart diseases and cancer [32]. Exogenous antioxidants intake would ameliorate the damage caused by oxidative stress [32,33]. Therefore, we checked the antioxidant activity of the aerial parts of *C. iphinoides* and it appeared that acetone extract had the strongest effect (IC_{50} 4.2 µg/ml), followed by aqueous (5.2 µg/ml) and methanol (7.5 µg/ml) extracts. These extracts had a strong antioxidant activity a little higher than antioxidant activity of trolox, the reference standard; however, hexane extract had a weak antioxidant effect (218 µg/ml). The IC_{50} we observed is higher than what was reported earlier in water and ethanol extracts (50 µg/ml) obtained from aerial parts of *C. iphinoides*, while hexane extract had very slight effect similar to our finding [24,34] Moreover our findings regarding methanol extract are in agreement with methanol derived from the shoots only investigated by Al-Mustafa et al. [35] however, IC_{50} we observed of water extract is higher than what was reported by Al-Mustafa et al. take into account that all of the above mentioned studies collected the plant from Jordan.

Cancer is disorder in which some cells loss the control on cell cycle [36]. It is considered the second cause of death all over the world [36, 37] and in Palestine [38]. Plants have been primary sources of anticancer agents, such as vinblastine, vincristine, etoposide, paclitaxel, docetaxel, topotecan, and irinotecan, which are among the most effective cancer chemotherapeutics currently available [39,40]. This prompted us to investigate the cytotoxic activities of aerial parts derived from *C. iphinoides* and it appeared that both hexane and acetone had a strong antiproliferative effect followed by methanol, while aqueous extract had no effect. antiproliferative effect could be due to a growth arrest, apoptosis or necrosis. The preferred mechanism of action for an anticancer agent is to trigger apoptosis system in cancer cells [32]. To reveal this, we investigated the effect of hexane extract on caspase-3 activity, as it was one of the strongest anti-proliferative stimulants. Interestingly, hexane extract had a stronger apoptotic effect than the chemotherapeutic medicine, doxorubicin. Caspase-3 is known as an executioner caspase in apoptosis because of its role in coordinating the destruction of cellular structures such as DNA fragmentation or degradation of cytoskeletal proteins [25]. Our data clearly suggest that *C. iphinoides* inhibits growth of cells through at least apoptosis and therefore it has an anticancer property. On the other hand the water extract has been shown to be safe to use. The cytotoxic properties observed in our study are in agreement with Abbas et al. and Al-Dabbas et al. [24,41]; however, they used the essential oils not the aerial parts and it was collected from Jordan. Halees et al. [42] showed that 300 mg/kg dichloromethane extract inhibited the cell growth of breast cancer cell lines in diabetic and non-diabetic mice. Unfortunately, there is not enough data and evidence on the safety, toxicology and lethality of this plant. It is not described in the databases of food and drug administration in USA and in European medicine agency. Recently, the median lethal dose has been investigated in mice and it appeared to be 750 mg/kg. Therefore there is a necessity to investigate the safety, toxicology and lethality thoroughly in the future.

Our study has few potential limitations. First, we did not screen the phytochemical composition of *c. iphinoides*. Second, fractionation of extracts, separation and identification of the bioactive compounds were not performed in the current study. However, this will be our priority in the future studies. Second, this is an *in vitro* study and our findings need an *in vivo* validation in the future.

5. Conclusion

In the current study we have found that acetone and hexane extracts possess a strong antiproliferative property followed by methanol extract whichwas been mediated via apoptosis; suggesting that they represent an attractive source of bioactive anticancer molecules. Acetone and hexane extracts possess strong and broad spectrum antimicrobial effects even stronger than standard antibiotics, followed by methanol extracts. Acetone extract has a strong antioxidant effect followed by methanol extract, while hexane extract has a weak effect. Interestingly, water extract has a strong antioxidant effect and anti- *S. sonnei* gram negative

bacteria, but it has no cytotoxic effect. This suggests that the water extract is a potential safe source of antioxidant agents and natural preservative instead of chemical preservatives in pharmaceutical and food products. However, it is necessary to validate these findings *in vivo* in the future.

CRediT authorship contribution statement

Reem Sbieh: Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Saad Al-Lahham:** Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Nidal Jaradat:** Data curation, Formal analysis, Resources, Writing – review & editing.

Declaration of Competing Interest

No conflict of interest was declared by the authors. Dr. Nidal Jaradat is an associate editor of the European Journal of Integrative Medicine.

Data availability

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

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