



## Antioxidant, antimicrobial, and cytotoxic properties of four different fractions derived from the bulbs of *Urginea maritima* L.

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### Abstract

**Introduction:** *Urginea maritima* L. (*U. maritima*) has been utilized in traditional medicine, but in many cases, it is not based on scientific evidence due to limited studies, particularly in Palestine. **Objective:** This study aimed to assess the antioxidant, antimicrobial, and cytotoxic properties of four solvents fractions extracted from *U. maritima* bulbs collected from Palestine for the first time. **Methods:** The DPPH method was used to quantify antioxidant activity. Antimicrobial activity was tested employing the broth microdilution method. Anti-proliferative activity was evaluated utilizing colorimetric methods. **Results:** The DPPH free radical scavenging assay revealed that the *U. maritima* aqueous and methanol fractions demonstrated no antioxidant properties. In contrast, acetone and hexane fractions exhibited significant activity with IC<sub>50</sub> values of 24 and 10.33 µg/mL, respectively. Acetone and hexane fractions had a broader spectrum of antimicrobial activity (ranging from 0.8 to 6.3 mg/mL) compared to aqueous and methanol fractions. Aqueous fractions reduced cell viability by 50% within 24-48 h. Methanol and acetone fractions reduced cell viability by 40% after 4 and 24 h (*p*-value <0.0001), while prolonged exposure to methanol, acetone, and hexane fractions for 48 h resulted in a substantial decrease in cell viability by 70% to 90% (*p*-value <0.0001). **Conclusion:** Our

findings have revealed that acetone and hexane fractions exhibited strong antioxidant, cytotoxic, and antimicrobial activities compared to the aqueous and methanol fractions. These observations offer significant insights into the potential therapeutic applications of *U. maritima* in combatting oxidative stress, microbial infections, and perhaps cancer. Further *in vivo* investigations are necessary to validate these findings in the future.

**Keywords:** *Urginea maritima*. Fractionation. Antioxidant. Cytotoxicity. Antimicrobial.

### Introduction

Plant-derived substances have long been utilized worldwide to treat, mitigate, or prevent several health ailments [1]. The diverse array of naturally derived biochemical compounds, whether obtained from botanical sources or in their pure state, offer several opportunities for developing novel pharmaceuticals [2]. This is clearly evidenced by the notion that almost half of the pharmaceutical products authorized by the United States Food and Drug Administration (FDA) over the period spanning from 1981 to 2014 have been either composed of natural substances or derived directly from them [3, 4].

In addition, approximately 60% of the global population relies on traditional medicine for their

healthcare needs, as reported by the World Health Organization (WHO) [5]. This highlights the significance of plant-derived medications in contemporary medical practice and emphasizes the need for more investigations and improvements. Palestine has a wide variety of plants; over 2700 various types are indigenous to this area [6]. Consequently, it is imperative to investigate the botanical specimens used in traditional medicine within this geographical region. Such an inquiry would enable the identification of their therapeutic advantages [7].

*Urginea maritima* L. commonly known as sea squill, is a flowering plant Indigenous to the Mediterranean basin, including Palestine, Northern Africa, the Middle East, and Europe [8]. The bulbs contain significant bioactive compounds, including cardiac glycosides, phenolic compounds, phytosterols, and other phytochemicals [9]. The species has been seen to exhibit successful growth in diverse soil compositions, including arid, humid, and rocky terrain [10]. Ingestion of substantial quantities of the desiccated bulb of this specimen may elicit potent physiological responses, such as diuretic, expectorant, cardiotoxic, and emetic effects [9, 11].

For this reason, it is used as a cardiotoxic diuretic in Europe to treat cardiac marasmus and edema [12]. Globally, *U. maritima* has been traditionally employed to treat various ailments, including chronic bronchitis, asthma, cardiac failure, and many other diseases [13]. In Palestine, the bulb of *U. maritima* has been used as a cardiotoxic, emetic, diuretic, as well as a mucolytic agent [14].

Additionally, to alleviate several skin disorders [15, 16]. However, the use of *U.*

*maritima* in Palestine tends to be based on traditional practice rather than scientific research due to the lack of investigations undertaken on this topic in Palestine. Keeping in mind that the composition and morphology of plants and consequently their potential biological properties are influenced by the ecological conditions of the region in which they grow. *U. maritima* that grows in Palestine may have distinct composition and properties compared to those examined in other geographical areas [17].

The global rise in antimicrobial resistance, along with the increased prevalence of oxidative stress-related diseases, and cancer [18], highlight the need to develop new therapeutic agents from botanical sources. *U. maritima* has been identified as a promising botanical agent, however, this plant is lacking research in Palestine. Hence, it is necessary to investigate the indigenous *U. maritima* L. specimens obtained from Palestine for the first time, assessing their antioxidant, antimicrobial, and cytotoxic attributes.

## Materials and Methods

### Collection and preparation of bulbs obtained from *U. maritima*

In October 2016, specimens of *U. maritima* bulbs were collected from the Kafr-Raie mountains in Jenin, Palestine. These samples were identified by Dr. Nidal Jaradat, a pharmacognosist, in the Pharmacognosy Laboratory at An-Najah National University. A voucher specimen (Pharm-PCT-2553) has been meticulously preserved in the herbarium of the Laboratory of Pharmacognosy. After being thoroughly washed with distilled water, the Bulbs were dried in the shade at room temperature (25°C) before their utilization and then they were kept in containers for future use.

### Fractionation Procedure

Using a mechanical grinder, dried bulbs of *U. maritima* were ground up in the laboratory, yielding a finely milled powder. The resultant powder was then carefully placed in airtight containers and labeled for future use. A fractional method was employed to obtain 50 g of this powder which was then sequentially mixed with 500 mL of solvents of varying polarities. The solvents employed in this procedure included hexane (a non-polar organic solvent), acetone (a mildly polar organic solvent), methanol (a highly polar organic solvent), and distilled water (an inorganic polar solvent). Following this, the plant fractions underwent agitation at a rate of 100 rounds per minute for 72 h at room temperature. After three days, each fraction was filtered using filter papers and a Buchner funnel. The organic fractions were subsequently filtered and concentrated under vacuum on a rotator evaporator device, whereas the aqueous fraction was dried through a freeze dryer. Finally, all the dried fractions were stored in a refrigerator for later use.

### *In vitro* evaluation of anti-oxidant activity via DPPH radical method

A solution with a concentration of 1 mg/mL was produced to assess the antioxidant capabilities of the fractions obtained from *U. maritima*. Afterward, the stock solution was used to create a series of working solutions with concentrations of 1, 2, 5, 10, 20, 50, and 100 µg/mL. Methanol was used as the solvent. A newly made DPPH solution with a concentration of 0.002% weight/volume was mixed with methanol, and each of the working concentrations had an equal ratio of 1:1:1. Methanol was used as a reference solution. The solutions were placed in a dark environment at room temperature for 30 min. The spectrophotometer was used to measure the absorbance values of the samples at a wavelength of 517 nm. The proportion of

antioxidant activity in each plant fraction and Trolox was calculated using a formula described in a previous study.

### Antimicrobial activity

Antibacterial activities of the four fractions were tested against six reference bacterial strains and two reference fungal strains acquired from the American Type Culture Collection (ATCC). The tested bacterial strains were *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Shigella sonnei* (ATCC 25931), *Enterococcus faecium* (ATCC 700221), *Staphylococcus aureus* (ATCC 25923) and Methicillin-Resistant *Staphylococcus aureus*. (MRSA) clinical isolate. The fungal strains that underwent testing were *Candida albicans* (ATCC 90028) and *Epidermatophyton floccosum* (ATCC 52066).

Solutions were prepared by dissolving 25 mg/mL of the hydrophilic and hydrophobic fractions in sterile distilled water and Dimethyl sulfoxide (DMSO), respectively. To achieve sterility, these solutions underwent sterilization using 0.45  $\mu\text{m}$  filters. Inoculated fresh bacterial isolates were mixed with sterile normal saline until they reached a turbidity level equivalent to the 0.5 McFarland standard corresponding to a concentration of  $1.5 \times 10^8$  CFU/mL. The suspension was subsequently diluted to achieve a final concentration of  $5 \times 10^5$  CFU/mL. The solutions of plant fractions were diluted with Mueller-Hinton II Broth in a serial two-fold manner, repeating the process 11 times. To serve as a positive control for microbial growth, Mueller-Hinton II broth devoid of plant fraction was employed. Wells were inoculated with or without various types of bacteria and fungi in duplicates. Inoculated plates were incubated at 35 °C for 24 h. The minimal inhibitory concentration (MIC) indicates the lowest concentration of plant fractions, which effectively inhibited visible microbial growth within the test broth.

### Cell culture and proliferation assay

The HeLa cervical cancer cell line was cultivated in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin antibiotics, and 1% l-glutamine. The cells were incubated in a humidified atmosphere with 5% CO<sub>2</sub> at 37 °C and were treated as described earlier [28]. Namely, cells were treated with 2.5, 1.25, 0.625, 0.3125, and 0.0 mg/mL of each fraction, followed by incubation periods spanning 4, 24, and 48 h. 10  $\mu\text{M}$  of doxorubicin, a chemotherapeutic medicine, was used as a positive control. The anti-proliferative property of the plant fractions was determined using the Cell Titer 96® Aqueous One Solution Cell Proliferation (MTS) assay

according to the manufacturer's instructions (Promega Corporation, Madison, WI).

### Statistical Analysis

The statistical analysis was carried out using GraphPad Prism 6.01 software. Group comparisons were conducted using the t-test, while the determination of the IC<sub>50</sub> was determined using a nonlinear regression analysis. Multiple group comparisons were executed using a two-way analysis of variance (ANOVA). Following the ANOVA, a Bonferroni post hoc test was done. The threshold for statistical significance was established at a p-value of 0.05.

## Results

### Antioxidant activity

Figure 1 illustrates the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activities induced by various concentrations of aqueous, methanol, acetone, and hexane fractions derived from the bulb of *U. maritima*. As shown in Table 1. Aqueous and methanol fractions exhibited very weak antioxidant activity, with IC<sub>50</sub> values of 319085 and 99842  $\mu\text{g/mL}$ , respectively. In contrast, IC<sub>50</sub> values of antioxidant activities of acetone and hexane fractions were 24 and 10.33  $\mu\text{g/mL}$ , respectively. The

IC<sub>50</sub> value of Trolox, the standard material, was 0.7  $\mu\text{g/mL}$ .

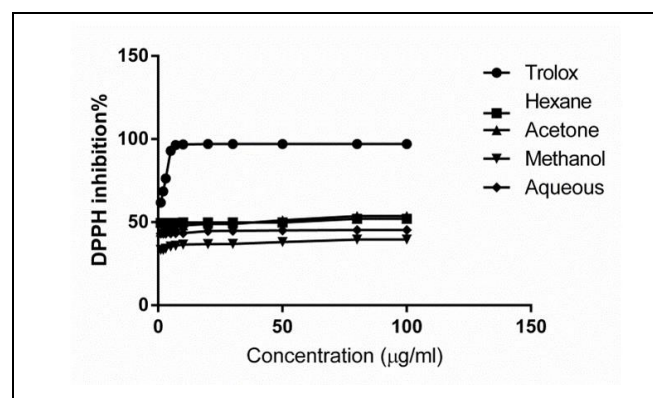


Figure 1. Dose-response inhibition of radical scavenging activity. 100, 80, 50, 40, 30, 20, 10, 7, 5, 3, 2, 1  $\mu\text{g/mL}$  of aqueous, methanol, acetone and hexane fractions derived from *U. maritima* were tested for radical scavenging activity employing DPPH method. Results were depicted as percentage of inhibition. Trolox was used as the reference standard. Source: Own authorship.

Table 1. IC<sub>50</sub> values of Trolox standard reference and fractions derived from *U. maritima*.

Fractions of <i>U. maritima</i> / reference standard	IC <sub>50</sub> $\mu\text{g/mL}$ (mean $\pm$ SE)	95% CI
Aqueous	319085 $\pm$ 2.5	56546 to 1.8*10 <sup>6</sup>
Methanol	99842 $\pm$ 3.2	9396 to 1*10 <sup>6</sup>

Acetone	24 ± 1.1	19 to 30.7
Hexane	10.33 ± 1.3	6 to 17.7
Trolox (reference standard)	0.73 ± 1.1	0.6 to 0.9

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

### Antimicrobial activity

As demonstrated in Table 2, aqueous and methanol fractions of *U. maritima* showed no antimicrobial activity against a panel of tested microbes except for MRSA with a MIC value equal to 6.3 mg/mL induced by aqueous fraction treatment only. Acetone fraction, on the other hand, exhibited antimicrobial activity against a broader range of microorganisms, including *S. aureus*, MRSA, *S. sonnie*, *P. aeruginosa*, *C. albicans* and *E. floccosum* with a MIC value of 6.3 mg/mL and against *E. faecium* with a MIC value of 0.8 mg/mL. Hexane fractions had potent antimicrobial activity against *S. aureus*, MRSA, *E. coli*, *S. sonnie*, *P. aeruginosa*, *C. albicans*, and *E. floccosum* with MIC values of 3, 6.3 1.6, 3, 1.6, 3 and 3 mg/mL, respectively.

Table 2. Antimicrobial activity of fractions derived from *U. maritima* determined by minimum inhibitory concentration method (mg/mL).

Bacterial species	MIC (mg/mL)			
	Aqueous	Methanol	Acetone	Hexane
<i>Staphylococcus aureus</i>	NA	NA	6.3	3
MRSA	6.3	NA	6.3	6.3
<i>Enterococcus faecium</i>	NA	NA	0.8	NA
<i>Escherichia coli</i>	NA	NA	NA	1.6
<i>Shigella sonnie</i>	NA	NA	6.3	3
<i>Pseudomonas aeruginosa</i>	NA	NA	6.3	1.6
<i>Candida albicans</i>	NA	NA	6.3	3
<i>Epidermatophyton floccosum</i>	NA	NA	6.3	3

NA: no activity. Source: Own authorship.

### Anti-proliferative activity

HeLa cervical adenocarcinoma cells were exposed to varying concentrations (0.0, 0.3125, 0.625, 1.25, and 2.5 mg/mL) of aqueous, methanol, acetone, and hexane fractions derived from the bulb of *U. maritima* over different time intervals: 4, 24, and 48 h. Aqueous fractions didn't exhibit any changes in cell viability after 4 hours. However, after 24 and 48 hours, cell viability was reduced by nearly 50%. Both Methanol and acetone fractions were found to inhibit cell viability (p-value <0,0001) after 4 and 24 hours, to approximately 40%, as depicted in Figure 2A and C. Figure 2B illustrates that treatment with hexane fraction for 24 h also led to a dose-dependent reduction

in cell viability (p<0.0001), ranging from 50% to 90%. Furthermore, treatment with methanol, acetone, and hexane fractions for 48 h across all tested concentrations inhibited (p-value <0.0001) cell viability in a dose-dependent manner, with reductions ranging from 70% to 90%, as illustrated in Figure 2A-C. As a positive control, treatment with doxorubicin resulted in a consistent decrease in cell viability to approximately 50% at all time points.

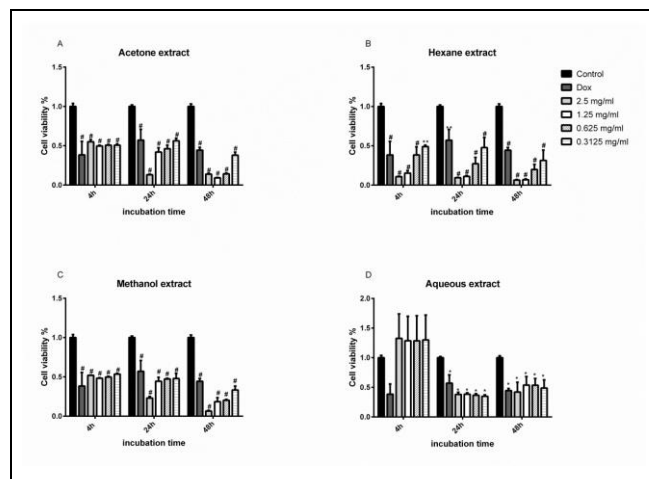


Figure 2. Anti-proliferative effect of (A) acetone, (B) hexane, (C) methanol, (D) and aqueous fractions obtained from *U. maritima*. HeLa cells were treated with 2.5, 1.25, .625 and .3125 mg/mL of acetone, hexane, methanol and aqueous fractions obtained from *U. maritima* and incubated for 4, 24 and 48 h. Proliferation was determined by MTS assay. Results were depicted as relative quantities (RQs) compared to the control (with only media; C). #p<0.0001, \*\*p<0.01 and \*p<0.05. Error bars represent SD. Source: Own authorship.

### Discussion

*U. maritima* is a plant that grows in Palestine and has a long history of utilization in Palestinian and global traditional medicine. However, insufficient comprehensive scientific evidence confirms the properties of *U. maritima* collected from Palestine. Considering that plants' composition and morphology and their potential biological properties are influenced by the ecological conditions of the region in which they grow [19,20]. Therefore, four fractions were derived from the bulb of *U. maritima*, which was collected from Jenin, Palestine. For the first time, the antioxidant, antibacterial, and cytotoxic characteristics of the *U. maritima* bulb cultivated in Palestine were investigated.

Reactive oxygen species (ROS) are radical species generated in living systems [21]. High levels of ROS can cause irreversible damage to biomolecules, such as nucleic acids, lipids, proteins, polyunsaturated fatty acids, and carbohydrates. Consequently, the

overproduction of ROS is strongly associated with various pathologic conditions such as cardiovascular and neurodegenerative diseases, atherosclerosis, and cancer [22]. These diseases are the leading causes of death worldwide. Various synthetic antioxidants have been invented and used to neutralize the oxidative stress to combat these diseases. However, these synthetic agents have dangerous side effects on human health [21, 22]. Therefore, we have investigated the antioxidant activities of fractions derived from an alternative natural source, *U. maritima* bulb. Based on our findings, the percentage of antioxidant activities varied depending on the tested fraction and concentration.

Aqueous and methanol fractions had no antioxidant characteristics; however, acetone and hexane fractions showed a significant antioxidant activity with IC<sub>50</sub> values of 24 and 10.33 µg/mL, respectively. This is in contrast to the limited number of studies that reported strong antioxidant effects of the methanolic and aqueous extracts derived from *U. maritima* bulbs collected from Algeria and Turkey. However, the acetone extract from the leaves of the plant exhibited the highest antioxidant activity [23, 24]. These discrepancies may be attributed to the limited number of studies on this subject, fractionation methods applied, and/or the difference in the soil and climates [19,20].

Cancer is the second cause of death all over the world [25]. For many years, numerous plant species and their secondary metabolites have been used for cancer treatment and prevention [26-28]. In our study, we examined the cytotoxic effects of *U. maritima* on HeLa cervical cancer cells. Our findings revealed that dose, duration, and the type of the tested fraction have all varying effects on the cytotoxic activity. Notably, The Hexane fraction had the most significant effect, followed by acetone, methanol, and aqueous fractions. This is in agreement with earlier studies, which have shown that various fractions from different parts of *U. maritima* have cytotoxic properties [29-31]. In addition, *U. maritima* has been shown to selectively inhibit the growth of colorectal cancer cell lines BY INDUCING APOPTOSIS [30]. *U. maritima* bulbs have been used as rodenticide, due to their poisonous effects on rats when ingested in high doses, with LD50s of 350 to 500 mg/Kg in male rats and 60 to 100 mg/ Kg in female rats, however, no toxic effect was detected on humans [32]. Altogether, it seems that fractions derived from the bulbs of *U. maritima* are safe and have a potential cytotoxic effect against cancer cell line specifically.

The use of antibiotics has played a crucial role in

reducing the spread and severity of numerous infectious diseases. However, such illnesses remain currently the second leading cause of death in several countries [18]. The misuse of antibiotics has led to widespread of antibiotic resistance worldwide, which poses a serious threat to the healthcare systems [29]. MRSA (Methicillin-resistant *S. aureus*), *P. aeruginosa*, *E. coli* and *Klebsiella* species are among the bacteria that exhibits antibiotic resistance [25]. To overcome this challenge, medicinal plants have been explored as alternative sources of natural antimicrobials which have gained significant attention [33-35]. In our study, we found that neither aqueous nor methanol fractions of *U. maritima* have any significant antimicrobial effects on the tested microbial strains, except for MRSA.

Hexane fraction exhibited antimicrobial and antifungal properties, followed closely by the acetone fraction. This is consistent with an earlier study that reported antifungal activity against *C. albicans* and *E. floccosum* [29]. Utilizing another part of the *U. maritima* (i.e. aerial parts) and other extraction methods have shown similar antimicrobial effect [36,37]. In contrast to our findings, methanolic and some polar organic solvents fractions derived from the root and the bulb, have shown antimicrobial effect [38-42]. The reason for the discrepancies may arise from various factors such as the tested part of the plant, the fractionation method, the climate, the soil and the tested microbes.

## Conclusion

It was revealed that the differential biological activities of fractions derived from the bulb of *U. maritima* L. Hexane fraction had the strongest antioxidant, antimicrobial and cytotoxic effects, followed by acetone, methanol, and aqueous fractions. Our findings suggest that *U. maritima* is a promising biological source for multiple applications such as medicine and food preservation and therefore, identifying these biomolecules specially in acetone fraction is necessary in the future. Furthermore, in vivo investigations are necessary to validate these findings in the future.

## CRedit

**Author contributions:** Saad Al-Lahham: Conceptualization, Visualization, Writing – original draft, Writing – review & editing, Investigation, Formal analysis, Data curation, Methodology. Nidal Jaradat: Conceptualization, Writing – review & editing, Investigation, Formal analysis, Data curation, Methodology. Other authors: Investigation, Data

curation, Formal analysis, Methodology, Writing – original draft. Wafa Jalil: Writing – review & editing and Data curation.

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## Ethical Approval

Not applicable.

## Informed Consent

Not applicable.

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## Data Sharing Statement

All data is contained within the article.

## Conflict of Interest

The authors declare no conflict of interest.

## Similarity Check

It was applied by Ithenticate®.

## Application of Artificial Intelligence (AI)

Not applicable.

## Peer Review Process

It was performed.

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