Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2019, Volume 10, Issue 11, Page 1053-1061

http://www.jmaterenvironsci.com

In vitro antimicrobial activity of *Urginea maritima* (L.) Baker bulb extract against food-borne pathogens

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Received 23 Aug 2019,
Revised 02 October 2019,
Accepted 04 October 2019

Keywords

- ✓ *Urginea maritima,*
- ✓ Plant extract,
- ✓ Food-borne pathogens,
- ✓ Antimicrobial activity.

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Abstract

Synthetic antimicrobial agents have been extensively used to protect food products but are now under debate due to the development of microbial resistance as well as human health concerns. In this context, plants emerge as a promising tool for microbial control in the food chain, and growing interest is being observed on the search for phytocompounds with these properties. The objectives of this study were to determinate the antimicrobial activity of *Urginea maritima* bulb extract. The antibacterial activity of the studied extract was evaluated against referenced bacteria *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae* using 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2Htetrazolium-bromide (MTT) assay. Moreover, its antifungal activity was investigated against *Sclerotium rolfsii*, *Fusarium oxysporum*, *Colletotrichum graminicola*, and *Penicillium digitatum* using the well agar diffusion test. The results showed that the tested extract was effective to control the growth of food-borne pathogen strains. In fact, *U. maritima* bulb extract exhibited a higher antifungal activity against *P. digitatum* (EC_{50} = 69.01 ± 2.29 µg/well) and *C. graminicola* (EC_{50} = 86.89 ± 1.17 µg/well). In addition, the extract under study possessed a high antibacterial activity against *S. aureus* (66.81 ± 1.06 %) and *B. subtilis* (57.94 ± 0.92 %). The findings of this work suggested that *U. maritima* bulb extract inhibited microbial growth through membrane damage.

1. Introduction

The food-borne pathogens contamination can occur at various stages in the food chain (e.g. in the field, during storage and processing) [1]. They are one of the major public concerns in both developed and developing countries [2]. In fact, they are considered as a major cause of infectious diseases outbreaks and accounted for considerably high cases of illnesses attacking human [3]. *Escherichia coli* is one of the main food-borne pathogens responsible for food poisoning and subsequent enteric infection [4]. In addition to bacteria, fungi have been recognized as the main cause of food deterioration during processing and storage and various human infections. Currently, there has been an increasing interest toward some fungi belonging to the genera *Penicillium* and *Fusarium*. The occurrence of these fungi in foodstuffs may represent a health hazard to humans due to the production of mycotoxins [5-6].

Therefore, a wide range of synthetic antimicrobial agents have been used for the eradication of these pathogens [7] to limit microbial growth in the food chain, to ensure that food products remain safe and unspoiled and to minimize human exposure to food-borne pathogens [8-9-10-11].

However, recurrent application over the years has led to the development of food-borne pathogens resistance. This phenomenon was considered as a major threat to public health [12]. Foods contamination with antimicrobial-resistant bacteria may occur at the primary production stage or at other stages in the supply chain. In fact, Akbar and Anal, (2013) [13] reported that *Salmonella* and *S. aureus* isolated from retail poultry meat were found resistant to various commercially available antibiotics. In addition, synthetic antimicrobial agents have been reported to have dangerous effects on human health due to their carcinogenic and teratogenic attributes as well as residual toxicity [14]. At the same time, there has been an increasing consumer demand for foods free or with low chemical substances because of their reported adverse effects.

All of the above concerns, many research efforts have been directed towards the progressive removal of chemicals substances and exploring new potent antimicrobial compounds [15]. Plants have emerged as a promising source and considerable works have been done to investigate novel antimicrobial compounds from plants, leading to the identification of phytocompounds as potential antimicrobial agents. In fact, plants synthesize an array of phytocompounds to protect themselves from microbial attacks and most of them are well known for their antimicrobial activities [16-17-18].

Urginea maritima (L.) Baker is a native medicinal and ornamental plant from the Mediterranean area. The bulbs have long been used in folk medicine as a source of natural products with pharmaceutical applications [19-20-21]. In addition, it is reported that plants belonging to the genus *Urginea* are a rich source of bioactive molecules. In this context, Maazoun *et al*., (2017) [22] showed that phenolic compounds formed *U. maritima* bulb extract, majorly. Indeed, *U. maritima* bulb extract was found to be rich in total phenolics (130.88 ± 0.44 mg GAE/g FW), flavonoids (50.81 ± 0.25 mg CE/g FW) and condensed tannins (6.76 ± 0.10 mg CE/g FW). In addition, the alkaloid content of *U. maritima* bulb extract was evaluated to be 12.09 ± 0.16 mg AE/g FW (Maazoun et *al*., 2017) [22]. On the other hand, Maazoun *et al*., (2018) [23] revealed that the *U. maritima* bulb extract exhibited a substantial antioxidant activity and an efficient capacity to inhibit α-amylase and acetylcholinesterase activities, suggesting its potential value as a source of natural antioxidants.

Thus, the objectives of this work were to assess the antimicrobial activity of *U. maritima* bulb extract against food-borne pathogens and to understand its mechanism of action by closely examining the interaction of the studied extract with bacterial and fungal cell membranes.

2. Material and Methods

2.1. Chemicals

All chemicals and solvents used were of analytical grade. Methanol, dimethyl sulfoxide (DMSO), azocasein, trichloroacetic acid (TCA), 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2Htetrazolium-bromide (MTT), benomyl, dichloran, streptomycin, O-nitrophenyl-β-D-galactoside (ONPG) and magnesium chloride ($Mgcl_2$) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Test microorganisms

The antibacterial activity of the bulb extract was tested against Gram-positive *Bacillus cereus ATCC 11778*, *Bacillus subtilis ATCC 11774*, *Staphylococcus aureus ATCC 6538*, *Listeria monocytogenes ATCC 19115* and Gram-negative *Escherichia coli ATCC 8739*, *Klebsiella pneumoniae ATCC 35657*, while the antifungal activity was evaluated against *Colletotrichum graminicola*, *Fusarium oxysporum*, *Sclerotium rolfsii* and *Penicillium digitatum*. The bacterial strains were provided by Laboratory of Protein Engineering and Bioactive Molecules, National Institute of Applied Sciences and Technology, Tunisia. The fungal strains were obtained from the Laboratory of Biotechnology Applied to Agriculture, National Agricultural Research Institute of Tunisia. The bacterial strains were subcultured on nutrient broth at 37 ºC, while the fungal strains were subcultured on Potato dextrose agar at 25 ºC.

2.3. Fungal protease

The fungal protease was provided by the Laboratory of Protein Engineering and Bioactive Molecules, National Institute of Applied Sciences and Technology (Tunisia).

2.4. Preparation of Urginea maritima bulb extract

Bulbs of *U. maritima* (L.) were collected from the region of Ras Jbel in the North East of Tunisia (37° 12′ 54″ North 10° 07′ 26″ East). The fresh bulbs were carefully washed with water. Then, grounded fresh bulbs (5 g) were extracted with 50 mL of methanol under the stirring condition for 24 h at room temperature. The macerate was centrifuged at 3500 rpm for 20 min. After that, the methanol was removed by evaporation to dryness under reduced pressure by a Rotavapor. The pellet was re-suspended in 1% DMSO for the evaluation of the antimicrobial activity.

2.5. Evaluation of antifungal activity

2.5.1. Mycelial growth inhibition assay

The evaluation of mycelial growth inhibition of *U. maritima* bulb extract was performed using the well agar diffusion test as reported by Mishra and Dubey, (1994) [24]. Briefly, a volume of 20 mL of the Potato Dextrose Agar (PDA) was poured into Petri plates (9 cm diameter) and allowed to solidify. PDA plates were inoculated by mycelial discs taken from 7 days old cultures with a sterile cork borer (4 mm diameter). A well (4 mm diameter)

was aseptically punched into the agar medium of each plate, and then filled with different volumes of the *U. maritima* bulb extract dissolved in 1% DMSO to obtain concentrations in the range of 20, 50 and 100 µg/well. Benomyl and 1% DMSO were used as positive and negative controls, respectively. The fungal colony diameters were recorded after incubation for 3, 6 and 9 days at 25 ± 2°C. The experiments were carried out in triplicate. Mycelial growth inhibition (MGI) was evaluated according to the following formula:

$$\text{MGI (\%)} = \frac{(\text{Dc}-\text{Dt})}{\text{Dc}} * 100$$

where MGI (%) = Mycelial Growth Inhibition, Dc (cm) = colony diameter of the control and Dt (cm) = colony diameter of the treatment.

2.5.2. Effect on Penicillium digitatum cell membranes integrity (release of cellular material)

This method is widely used to determinate the effect of antimicrobial agents on fungal membrane integrity (Paul *et al*., 2011) [25]. Three mycelial discs of *P. digitatum* (4 mm diameter) were taken from 7 days old culture and mixed with 100 mL of PDB. After that, *P. digitatum* cells were harvested by centrifugation at 10000 rpm for 10 min and resuspended in 20 mL PBS buffer (pH 7). After 30, 60, 90 and 120 min of incubation with *U. maritima* bulb extract, samples were centrifuged at 3000 rpm for 20 min. The UV (260 nm) absorbing materials in the supernatant was measured. Dichloran was used as a reference. The release of cellular material was estimated using the following equation:

$$\text{Release of cellular material (\%)} = \frac{(\text{ODc}-\text{ODt})}{\text{ODc}} * 100$$

where ODc was the absorbance of the control and ODt was the absorbance of the treatment.

2.5.3. Penicillium digitatum protease activity inhibition

This test was performed using the method of Belhadj *et al*., (2016) [26]. Briefly, a volume of 50 µL of *P. digitatum* protease (11.84 U/mL) was mixed with 20 µL of *U. maritima* bulb extract dissolved in 1% DMSO. The mixture was incubated for 10 min at 55°C. Next, 50 µL of azocasein solution (50 mg/ml) in the presence of 100 µL of Tris HCl buffer (100 mM, pH 7) was added. The mixture was incubated for another 10 min at 55 °C. Then, 600 µL of 10% TCA were added. An incubation on ice for 10 min was required before centrifugation of 13000 rpm for 5 min. A volume equivalent to 600 µL of the supernatant was mixed with 700 µL of sodium hydroxide (1 M). Absorbance was evaluated at 440 nm. Three tests were performed for each sample. Protease activity inhibition was evaluated according to the following formula:

$$\text{Protease activity inhibition (\%)} = \frac{(\text{Acontrol}-\text{Asample})}{\text{Acontrol}} * 100$$

where Acontrol = the absorbance of the control and Asample = the absorbance of the sample.

2.6. Evaluation of antibacterial activity

2.6.1. Bacterial growth inhibition (MTT assay)

Evaluation of the bacterial growth inhibition of *U. maritima* bulb extract was performed using MTT assay as reported by Wang *et al*., (2010) [27]. MTT is a water-soluble yellow dye that can be reduced to water-insoluble purple formazan crystals by the dehydrogenase system of active cells (Seidl and Zinkernagel, 2013). The formazan crystals formed can be quantified spectrophotometrically, and the OD values are directly proportional to the number of metabolically active cells in the culture. Briefly, 200 µL of a fresh cell suspension (10^7 CFU/mL) from logarithmic phase bacteria, was mixed with 100 µL of *U. maritima* bulb extract dissolved in 1% DMSO (1 mg/mL). Then, the mixture was incubated for 24 h at 37°C. After incubation, a volume of 25 µL of MTT solution (5 mg/mL) was added and the solution was incubated for 20 min at 37°C. Then, centrifugation at 3000 rpm for 10 min was required. A volume of 1% DMSO was added to the pellet for dissolution of formazan crystals. Bacterial growth was determined by optical density reading at 550 nm. Streptomycin was used as a reference.
Bacterial growth inhibition (BGI) was estimated using the following equation:

$$\text{BGI (\%)} = \frac{(\text{ODc}-\text{ODt})}{\text{ODc}} * 100$$

where ODc was the absorbance of the control and ODt was the absorbance of the treatment.

2.6.2. Effect on Escherichia coli cell inner membranes (β-galactosidase assay)

Inner membrane permeabilization was determined by measuring the release of cytoplasmic β-galactosidase activity from *E. coli* cells into the culture medium using O-nitrophenyl-β-D-galactoside (ONPG) as the substrate (Je and Kim, 2006) [28]. The bacterial suspension (10^7 CFU/mL) from logarithmic phase bacteria (200 µL) was incubated with 100 µL of *U. maritima* bulb extract (1 mg/mL) for 10-100 min at 30 °C. Centrifugation of 10 min at 11000 rpm was required to eliminate bacterial cells. Then, a volume of 10 µL of ONPG (30 mM) was mixed with 950 µL of phosphate buffer (50 mM pH 6.8) in the presence of $MgCl_2$ (1 mM). After that, the supernatant (50 µL) was added to the mix. Incubation of 10 min at 30°C was required to the enzymatic reaction. Finally, a volume of 250 µL of (Na_2CO_3 1M, EDTA 10 mM) was added to stop the reaction. The production of O-nitrophenol was evaluated by measuring absorbance at 420 nm. Streptomycin was used as a reference.

2.7. Statistical analysis

Results in tables and graphs were presented as the mean ± standard deviation (SD). All experimental results were submitted to one-way analysis of variance (ANOVA) using the SPSS software version 20.0. Values were considered significantly different according to the Tukey test at $P = 0.05$. The median effective concentration (EC_{50}) was determined by the regression analysis when the growth was reduced to 50% of the control. The median inhibitory concentration (IC_{50}) was calculated by the Graph Pad Prism 6.07 software program.

3. Results and discussion

The results revealed the antifungal potential of *U. maritima* bulb extract. In fact, the extract under investigation inhibited the mycelial growth of all tested fungi. Furthermore, the inhibition effect of the *U. maritima* bulb extract on fungi mycelial growth showed a concentration-dependent activity that can be evaluated by the determination of the EC_{50} values corresponding to the concentration of the extract required to reduce 50% of fungi mycelial growth. Low EC_{50} values indicate high antifungal activity. As shown in Table 1, *U. maritima* bulb extract exhibited the higher antifungal activity against *P. digitatum* and *C. graminicola* with an EC_{50} estimated to be 69.01 ± 2.29 and 86.89 ± 1.17 µg/well, respectively, followed by that of *S. rolfsii* (EC_{50} of 125.49 ± 1.36 µg/well). *F. oxysporum* was less sensitive to *U. maritima* bulb extract with an EC_{50} evaluated to be 208.48 ± 3.28 µg/well because of its ability to metabolize antifungal agents according to Vio-michaelis *et al*., (2012) [29].

Table 1: EC_{50} values of *Urginea maritima* bulb extract against pathogenic fungi

Fungal strain	*U. maritima* bulb extract	Benomyl
Fusarium oxysporum	208.48 ± 3.28 c	65.48 ± 1.36 c
Sclerotium rolfsii	125.49 ± 1.36 b	29.20 ± 1.42 ab
Colletotrichum graminicola	86.89 ± 1.17 ab	35.89 ± 0.64 b
Penicillium digitatum	69.01 ± 2.29 a	13.14 ± 0.90 a

Unit EC_{50} = µg/well, evaluated after 9 days at 25 ± 2°C; within rows, values followed by different letters denote significant differences at $P = 0.05$ according to Tukey test.

The effectiveness of *U. maritima* bulb extract to control the growth of fungal strains can be primarily attributed to its high phenolic and alkaloid contents as reported by Maazoun *et al*., (2017) [22]. Indeed, the inhibitory effect of the phenolic compounds on the fungi growth have been extensively reported [30-31-32-33]. In addition, numerous works highlighted the antifungal potential of alkaloids. In this regard, Singh *et al*., (2000) [34] reported the antifungal effect of an alkaloid isolated from *Alstonia venenata*. In addition, Singh *et al*., (2007) [35] isolated an alkaloid from *Phyllanthus amarus* (L.) and reported its antifungal activity against some fungi. More recently, Singburaudom, (2015) [36] demonstrated that the alkaloid berberine isolated from *Coscinium fenestratum* is an effective inhibitor of phytopathogenic fungi.

The effect of *U. maritima* bulb extract on *P. digitatum* cell membranes integrity was evaluated by measuring the release of 260 nm absorbing materials. In fact, leakage of intracellular constituents has been considered as a good indicator of loss of membrane integrity (Paul *et al*., 2011) [25]. Small ions such as potassium and phosphate tend to reach out first, followed by large molecules such as DNA, RNA, and other materials. Since these

compounds have strong UV absorption at 260 nm, they are described as 260 nm absorbing materials as reported by Paul *et al*., (2011) [25]. Data demonstrated that increased period of exposure to *U. maritima* bulb extract increased significantly the release of cellular material from *P. digitatum* cells, which could be considered as an indicator of membrane damage according to Chen and Cooper (2002) [37]. The release of cellular material from *P. digitatum* cells treated with *U. maritima* bulb extract for 120 min reached 28% and was higher than that of negative control (Figure 1). The phenolic compounds exhibited their antifungal potential at the level of the membrane according to Beltrame *et al*., (1988) [38] and affected fungi membranes integrity [39-40].

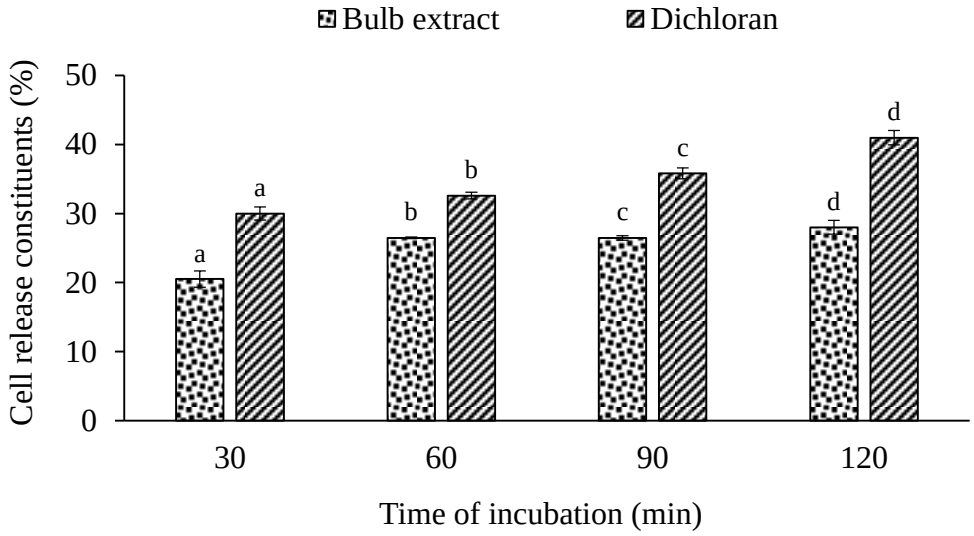

Figure 1: Effect of *Urginea maritima* bulb extract on the 260 nm-absorbing material release from *Penicillium digitatum* cells. Symbols with different letters indicate significant differences between times at $P = 0.05$ according to Tukey test.

Many species of pathogenic fungi secrete proteases during the contamination process. These proteolytic enzymes are involved in many aspects of foodstuffs contamination, especially during the degradation of food proteins. Their involvement in diverse aspects of food chain contamination makes them potential targets for fungi control [41-42-43]. *U. maritima* bulb extract was screened for its inhibition power against *P. digitatum* protease activity in order to search for effective fungi protease inhibitors from natural sources. The results showed that *U. maritima* bulb extract inhibited significantly the enzymatic activity of the tested protease. Moreover, a positive correlation between the concentrations of the studied extract and the protease activity inhibition was recorded ($r = 0.84$, $P < 0.05$). At a concentration of 500 µg/mL, the inhibition power of fungal protease activity was estimated to be 70.41 ± 1.84%. An IC_{50} in the range of 121.97 ± 1.22 µg/mL was required to ensure 50% of the fungal protease activity inhibition. These results indicated that *U. maritima* bulb extract contained protease activity inhibitors, which could probably bind to protease active site and form an inactive complex. Da Cruz Carbal *et al*., (2013) [44] explained that the OH groups of phenolic compounds could be able to form hydrogen bonds that have inhibitory effects on fungal enzymes activity.

The antibacterial activity of *U. maritima* bulb extract was quantitatively assessed by MTT assay. The cleavage of the tetrazolium salt MTT into a blue colored product (formazan) by the bacterial dehydrogenase system is potentially very useful for assaying bacterial growth (Wang *et al*., 2010) [27]. The amount of formazan produced is proportional to bacterial growth. As demonstrated in Table 2, *U. maritima* bulb extract presented antibacterial activity against both Gram-positive and Gram-negative bacteria. The extract under study possessed a high antibacterial activity against *Staphylococcus aureus* (66.81 ± 1.06 %) followed by *Bacillus subtilis* (57.94 ± 0.92 %). However, *Klebsiella pneumoniae* was found to be less susceptible to the extract under investigation (20.78 ± 0.60 %). Therefore, it may be postulated that the antibacterial activity of *U. maritima* bulb extract against the tested bacteria was associated with the Gram-positive and Gram-negative classification. In fact, Gram-negative bacteria were less susceptible to the action of the *U. maritima* bulb extract, presenting lower BGI values, which may be caused by the outer membrane of Gram-negative bacteria that act as a strong permeability barrier [45].

Table 2: Effect of *Urginea maritima* bulb extract on bacterial growth of food-borne pathogen bacteria

Bacterial strain	*U. maritima* bulb extract	Streptomycin
Listeria monocytogenes	49.60 ± 0.52 c[1]	80.15 ± 0.57 f
Bacillus cereus	55.46 ± 0.40 d	72.27 ± 1.20 c
Escherichia coli	38.46 ± 0,45 b	68.42 ± 0.45 b
Bacillus subtilis	57.94 ± 0.92 e	76.32 ± 1.15 d
Staphylococcus aureus	66.81 ± 1.06 f	79.66 ± 0.98 e
Klebsiella pneumoniae	20.78 ± 0.60 a	64.60 ± 0.18 a

[1]: Within rows comparisons were made between bacterial strain as a function of growth inhibition; BGI (Bacterial Growth Inhibition) followed by different letter are significantly different according to Tukey test at $P = 0.05$.

The effectiveness of *U. maritima* bulb extract to control the growth of bacterial strains can be attributed to its high phenolic content as reported by Maazoun *et al*., (2017) [22]. Indeed numerous studies revealed the antibacterial potential of phenolic compounds [46-47-48-49-50]. In addition, alkaloids, reported in *U. maritima* bulb extract by Maazoun *et al*., (2017) [22], may be involved in the antibacterial potential of the tested extract. In fact, alkaloids have been reported to be potent inhibitors of bacterial growth. In this context, Karou *et al*., (2005) [51] reported the antibacterial effect of alkaloids from *Sida acuta* (Magnoliopsida: Malvaceae). Similarly, Hymete *et al*., (2005) [52] isolated alkaloids from *Echinops ellebeckii* (Magnoliopsida: Chenopodiaceae) against *Staphylococcus aureus*. Inner membranes permeabilization of *E. coli* was evaluated as a function of cytoplasmic β-galactosidase release. Destabilization of the outer membranes is necessary to gain access to the inner membranes (Je and Kim, 2006) [28]. The increased release of cytoplasmic β-galactosidase caused by *U. maritima* bulb extract indicated that the permeability of inner membranes had increased. Therefore, *U. maritima* bulb extract seemed to be able to interact with inner membranes components such as lipids, leading to membranes permeabilization. As illustrated in Figure 2, the release of cytoplasmic β-galactosidase in *E. coli* suspension was increased time-dependently in the presence of *U. maritima* bulb extract ($F = 81.23$, $df = 5$, $P < 0.001$). In addition, the absorbance values of suspensions treated with *U. maritima* bulb extract were higher than those of negative control.

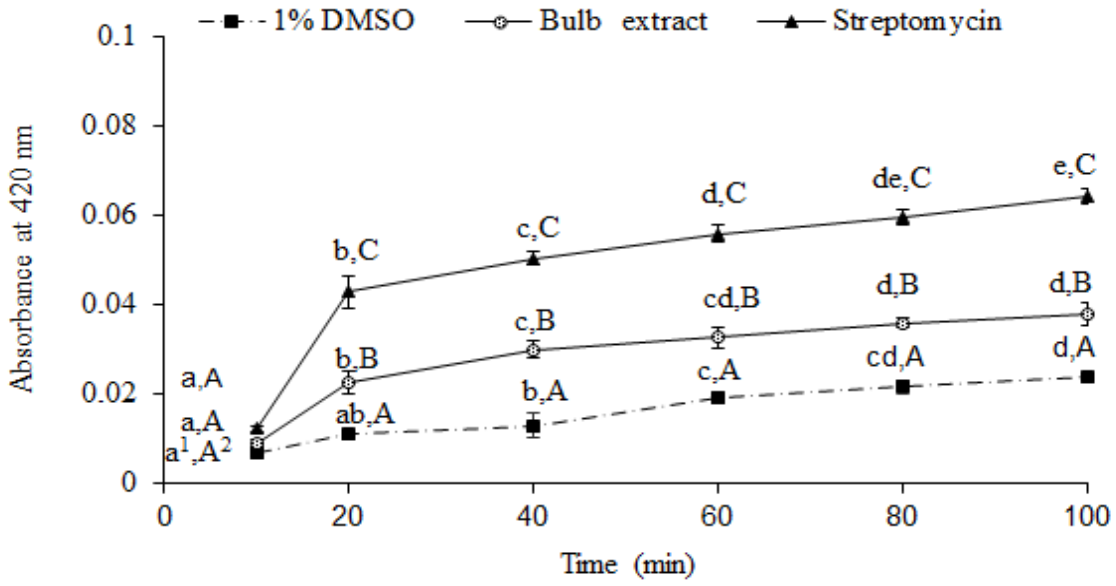

Figure 2: Effect of *Urginea maritima* bulb extract on *Escherichia coli* cell inner membranes; [1]: statistical differences have been done within treatments and marked by different letters according to Tukey test at $P = 0.05$; [2]: statistical differences have been done within treatments and marked by different letters according to Tukey test at $P = 0.05$.

Based on these findings, it may be suggested that *U. maritima* bulb extract inhibited bacterial growth by membranes permeabilization. The bacterial membranes undoubtedly are the target of many antimicrobial agents. When antimicrobial agents interact with bacterial membranes, they cause fundamental changes in bacterial membrane and function. In fact, when bacterial membranes become compromised by interaction with antimicrobial agents, intracellular constituents tend to leach (Denyer, 1990) [53]. The release of cytoplasmic β-galactosidase revealed inner membrane permeabilization of *E. coli* cells in the presence of *U. maritima* bulb extract. In addition, destabilization of the outer membranes is necessary to gain access to the inner membranes according to Je and Kim, (2006) [28]. Therefore, the extract under investigation seemed to be able to interact with bacterial membranes leading to their permeabilization.

Conclusion

Because of the adverse effects of synthetic antimicrobials and the emergence of microbial resistance, the phytocompounds obtained in the form of extract or fraction, have gained the considerable interest of researchers. This study indicated that *U. maritima* bulb extract exhibited a prominent antimicrobial activity against most referenced bacterial and fungal strains. Thus, considering these outcomes *U. maritima* bulb extract could be considered as a good source of antimicrobial agents. The broad spectrum of activity and natural origin of antimicrobial agents from *U. maritima* bulb extract make them attractive for various applications in medicine, agriculture, and food preservation, especially where the use of conventional antimicrobial agents is undesirable or prohibited. Further, *in vivo* studies are required to confirm the antimicrobial activity of *U. maritima* bulb extract.

Acknowledgments

The authors are very grateful to Laboratory of Biotechnology Applied to Agriculture, National Institute of Agricultural Research of Tunisia (INRAT), University of Carthage for supports in this study.

References

1. J.W. Bennett, M. Klich, Mycotoxins, *Clin. Microbiol. Rev*. 16 (2003) 497-516. https://doi.org/10.1128/CMR.16.3.497-516.2003
2. A.A. Tayel, W.F. El-Tras, Anticandidal activity of pomegranate peel extract aerosol as an applicable sanitizing method, *Mycoses* 53 (2010) 117-122. https://doi.org/10.1111/j.1439-0507.2008.01681.x
3. G. Chao, X. Zhou, X. Jiao, X. Qian, L. Xu, D. Savoia, Prevalence and antimicrobial resistance of foodborne pathogens isolated from food products in China, *Foodborne Pathog. Dis.* 4(2007) 277-284. https://doi.org/10.1089/fpd.2007.0088
4. J.C. Costa, F. Espeschit Ide, F.A. Pieri, A. Benjamin, M.A. Moreira, Increased production of biofilms by *Escherichia coli* in the presence of enrofloxacin, *Vet. Microbiol*. 160(2012) 488-490. https://doi.org/10.1016/j.vetmic.2012.05.036
5. M.E. Zain, Impact of mycotoxins on humans and animals, *J. Saudi Chem. Soc*. 15(2011) 129-144. https://doi.org/10.1016/j.jscs.2010.06.006
6. B. Kabak, A.D.W. Dobson, Biological strategies to counteract the effects of mycotoxins, *J. Food. Prot. 72(2009) 2006-2016.* https://doi.org/10.4315/0362-028X-72.9.2006
7. M. Friedman, P.R. Henika, R.E. Mandrell, Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*, *J. Food. Prot.* 65(2002) 1545-1560. https://doi.org/10.4315/0362-028X-65.10.1545
8. N. Boyraz, M. Ozcan, Antifungal effect of some spice hydrosols, *Fitoterapia* 76(2005) 661-665. https://doi.org/10.1016/j.fitote.2005.08.016
9. J. Varga, B. Tóth, Novel strategies to control mycotoxins in feeds, *Acta Vet. Hung*. 53(2005) 189-203. https://doi.org/10.1556/AVet.53.2005.2.4
10. B. Kabak, A.D.W. Dobson, I. Var, Strategies to prevent mycotoxin contamination of food and animal feed: a review, *Crit. Rev. Food Sci. Nutr.* 46(2006): 593- 619. https://doi.org/10.1080/10408390500436185
11. L. Natta, Essential oil from five *Zingiberaceae* for anti food-borne bacteria, *Int. Food. Res J.* 15(2008) 337-346.
12. M.A. Hussain, Antimicrobial resistance-bacteria in food products, *Adv. Food. & Nutr. Sci*. 2(2016): e1-e2. https://doi.org/ 10.17140/AFTNSOJ-2-e008

13. A. Akbar, A.K. Anal, Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat, *Asian Pac. J. Trop. Biomed.* 3(2013): 163-168. https://doi.org/ 10.1016/S2221-1691(13)60043-X
14. R.K. Pundir, P. Jai, C. Sharma, Antimicrobial activity of ethanolic extracts of *Syzygium aromaticum* and *Allium sativum* against food associated bacteria and fungi, *Ethnobotanical Leaflets* 2010(2010) 344-360.
15. C. Agatemor, Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. *EJE AFChe*. 8 (2009) 195-200.
16. E.M. Abdallah, Plants: an alternative source for antimicrobials, *J. Appl. Pharm. Sci.* 01 (2011) 16-20.
17. D. Savoia, Plant-derived antimicrobial compounds: alternatives to antibiotics, *Future Microbiol.* 7 (2012) 979-990. https://doi.org/ 10.2217/fmb.12.68
18. K.L. Compean, R.A. Ynalvez, Antimicrobial activity of plant secondary metabolites: a review, *J. Med. Plants Res.* 8 (2014) 204-213. https://doi.org/ 10.3923/rjmp.2014.204.213
19. A.L. Cogne, A. Marston, S. Mavi, K. Hostettmann, Study of two plants used in traditional medicine in Zimbabwe for skin problems and rheumatism: *Dioscorea sylvatica* and *Urginea altissima*, *J. Ethnopharmacol.* 75(2001) 51-53. https://doi.org/10.1016/S0378-8741(00)00347-0
20. T. Pohl, C. Koorbanally, N.R. Crouch, D.A. Mulholland, Bufadienolides from *Drimia robusta* and *Urginea altissima* (Hyacinthaceae), *Phytochemistry* 58 (4) 557-561. https://doi.org/10.1016/S0031-9422(01)00225-4
21. M. Adams, C. Berset, M. Kessler M, M. Hamburger, Medicinal herbs for the treatment of rheumatic disorders--a survey of European herbals from the 16th and 17th century, *J. Ethnopharmacol.* 121 (2008) 343-359. https://doi.org/10.1016/j.jep.2008.11.010
22. A.M. Maazoun, T. Ben Hlel, S.H. Haouel, F. Belhadj, J. Mediouni Ben Jemâa, M.N. Marzouki, Screening for insecticidal potential and acetylcholinesterase activity inhibition of *Urginea maritima* bulbs extract for the control of *Sitophilus oryzae* (L.), *J. Asia. Pacific. Entomol.* 20 (2017) 752-760. https://doi.org/10.1016/j.aspen.2017.04.004
23. A.M. Maazoun, F. Belhadj, J.M. Ben Jemâa, M.N. Marzouki, assessment of antioxidant potential and α-amylase and acetylcholinesterase inhibitory activities of *Urginea maritima* (L.) Baker bulbs, *J. Mater. Environ. Sci.* 9 (2018) 3197-3205.
24. A.K. Mishra, N.K. Dubey, Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities, *Appl. Environ. Microbiol.* 60 (1994) 1101-1105.
25. S. Paul, R.C. Dubey, D.K. Maheswari, S.C. Kang, *Rachyspermum ammi* (L.) fruit essential oil influencing on membrane permeability and surface characteristics in inhibiting food-borne pathogens, *Food Control* 22 (2011) 725-731. https://doi.org/10.1016/j.foodcont.2010.11.003
26. F. Belhadj, I. Somrani, N. Aissaoui, C. Messaoud, M. Boussaid, M. N. Marzouki, Bioactive compounds contents, antioxidant and antimicrobial activities during ripening of *Prunus persica* L. varieties from the North West of Tunisia, *Food Chem*. 204 (2016) 29-36. https://doi.org/10.1016/j.foodchem.2016.02.111
27. H. Wang, H. Cheng, F. Wang, D. Wei, X. Wang, An improved 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay for evaluating the viability of *Escherichia coli* cells, *J. Microbiol. Meth.* 82(2010) 330-333. https://doi.org/10.1016/j.mimet.2010.06.014
28. J.Y. Je, S.K Kim, Antimicrobial action of novel chitin derivative, *Biochim. Biophys. Acta* 1760 (2006) 104-109. https://doi.org/10.1016/j.bbagen.2005.09.012
29. S. Vio-michaelis, G. Apablaza-Hidalgo, M. Gómez, R. Peña-Vera, G. Montenegro, Antifungal activity of three Chilean plant extracts on *Botrytis cinerea*, *Bot. Sci.* 90 (2012) 179-183. http://dx.doi.org/10.17129/botsci.482
30. V. Lattanzio, D. De Cicco, G. Di Venere, A. Lima, M. Salerno, Antifungal activity of 2,5-dimethoxybenzoic acid on postharvest pathogens of strawberry fruits, *Ital. J. Food. Sci.* 1 (1994) 23-30. https://doi.org/10.1016/S0925-5214(96)00031-2
31. M.M. Cowan, Plant products as antimicrobial agents, *Clin. Microbiol. Rev.* 12 (1999) 564-582.
32. S.M. Al-Reza, A. Rahman, Y. Ahmed, S. C. Kang, Inhibition of plant pathogens *in vitro* and *in vivo* with essential oil and organic extracts of *Cestrum nocturnum* L., *Pestic. Biochem. Physiol.* 96 (2010): 86-92. https://doi.org/10.1016/j.pestbp.2009.09.005
33. D.D. Costa Carvalho, E. Alves, R. Barbosa Camargos, D. Ferreira Oliveira, J.R. Soares Scolforo, D.A. De Carvalho, T.R. Sâmia Batista, Plant extracts to control *Alternaria alternata* in *Murcott tangor* fruits, *Rev. Iberoam. Micol.* 28 (2011) 173-178. https://doi.org/10.1016/j.riam.2011.05.001
34. U.P. Singh, B.K. Sarma, P.K. Mishra, A.B. Ray, Antifungal activity of venenatine, an indole alkaloid isolated from *Alstonia venenata*, *Folia Microbiol.* 45(2000) 173-176. https://doi.org/10.1007/BF02817419
35. A.K. Singh, M.B. Pandey, U.P. Singh, Antifungal activity of an alkaloid allosecurinine against Some Fungi, *Mycobiology* 35 (2007) 62-64. https://doi.org/10.4489/MYCO.2007.35.2.062

36. N. Singburaudom, Hydroxychavicol from *Piper betel* leave is an antifungal activity against plant pathogenic fungi, *J. Biopesticides* 8 (2015) 28-36.
37. C.Z. Chen, S.L. Cooper, Interactions between dendrimer biocides and bacterial membranes, *Biomaterials*, 23(2002) 3359-3368. https://doi.org/10.1016/S0142-9612(02)00036-4
38. P. Beltrame, P.L. Beltrame, P. Carniti, D. Guardione, C. Lanzetta, Inhibiting action of chlorophenols on biodegradation of phenol and its correlation with structural properties of inhibitors, *Biotechnol. Bioeng.* 31 (1988) 821-828. https://doi.org/10.1002/bit.260310809
39. H. Ikigai, T. Nakae, Y. Hara, T. Shimamura, Bactericidal catechins damage the lipid bilayer, *Biochim. Biophys. Acta* 1147(1993) 132-136. https://doi.org/10.1016/0005-2736(93)90323-R
40. J. Terao, M. Piskula, Q. Yao, Protective Effect of Epicatechin, Epicatechin Gallate, and Quercetin on Lipid Peroxidation in Phospholipid Bilayers, *Arch. Biochem. Biophys.* 308 (1994) 278-284. https://doi.org/10.1006/abbi.1994.1039
41. M.B. Rao, A.M. Tanksale, M.S. Ghatge, V.V. Deshpande, Molecular and biotechnological aspects of microbial proteases, *Microbiol. Mol. Biol. Rev.* 62 (1998) 597-635.
42. M. Monod, S. Capoccia, B. Léchenne, C. Zaugg, M. Holdom, O. Jousson, Secreted proteases from pathogenic fungi, *Int. J. Med. Microbiol.* 292 (2002) 405-419.
43. I. Yike, Fungal proteases and their pathophysiological effects, *Mycopathologia* 171 (2011) 299-323. https://doi.org/10.1007/s11046-010-9386-2
44. L. Da Cruz Cabral, V. Fernández Pinto, A. Patriarca, Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods, *Int. J. Food Microbiol.* 166 (2013) 1-14. https://doi.org/10.1016/j., ijfoodmicro.2013.05.026
45. A. Smith-Palmer, J. Stewart, L. Fyfe, Antimicrobial properties of plant essential oils and essences against five important food- borne pathogens, *Lett. Appl. Microbiol.* 26 (1998) 118-122. https://doi.org/10.1046/j.1472-765X.1998.00303.x
46. R. Paolillo, C.R. Carratelli, A. Rizzo, Effect of resveratrol and quercetin in experimental infection by *Salmonella enterica serovar Typhimurium*, *Int. Immunopharmacol.* 11 (2011) 149-156. https://doi.org/10.1016/j.intimp.2010.10.019
47. A. Borges, C. Ferreira, M.J. Saavedra, M. Simoes, Antibacterial activity and mode of action of ferulic and gallic acids Against pathogenic bacteria, *Microb. Drug. Resist.* 19 (2013) 1-10. https://doi.org/10.1089/mdr.2a12.0244
48. M.G. Jinukuti, A. Giri, Antimicrobial activity of aqueous extract of *Terminalia chebula* Retz, *Recent Res. Sci. Technol*. 5 (2013) 46-49.
49. A. Karunanidhi, R. Thomas, A. Van Belkum, V. Neela, *In vitro* antibacterial and antibiofilm activities of chlorogenic acid against clinical isolates of *Stenotrophomonas* maltophilia including the trimethoprim/sulfamethoxazole resistant strain, *BioMed. Res. Int.* (2013) 1-7. http://dx.doi.org/10.1155/2013/392058
50. N. Kumar, V. Pruthi, Potential applications of ferulic acid from natural sources, *Biotechnol. Rep.* 4 (2014) 86-93. https://doi.org/10.1016/j.btre.2014.09.002
51. D. Karou, A. Savadogo, A. Canini, S. Yameogo, C. Montesano, J. Simpore, V. Colizzi, A.S. Traore, Antibacterial activity of alkaloids from *Sida acuta*, *Afr. J. Biotechnol*. 4(2005) 1452-1457.
52. A. Hymete, T.H. Iversen, J. Rohloff, B. Erko, Screening of *Echinops ellenbeckii* and *Echinops longisetus* for biological activities and chemical constituents, *Phytomedicine* 12 (2005) 675–679. doi.org/10.1016/j.phymed.2004.01.013
53. S.P. Denyer, G.S.A.B. Stewart, Mechanisms of action of disinfectants, *Int. Biodeter. Biodegr*. 26 (1990) 89-100. https://doi.org/10.1016/S0964-8305(98)00023-7