Jordan Journal of Pharmaceutical Sciences, Volume 16, No. 1, 2023

Osmotic Stress Enhances Antimicrobial Activity of in Vitro Grown Microshoots of *Ochradenus Baccatus* Delile Against Selected microbes

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ABSTRACT

Ochradenus baccatus Delile is a wild medicinal plant that produces several natural compounds with medicinal benefits. In this study, microshoots of *Ochradenus baccatus* were exposed to osmotic stress conditions consisting of Murashige and Skoog solid media (MS) media that containing different osmotic agents (sugar types) at a range of concentrations (including 0.1, 0.2, 0.3 or 0.4 M). The purpose was to test their effects on microshoots' growth and antimicrobial activities against selected strains of bacteria and one strain of fungi. It has been found that growth parameters (including shoot length and proliferation) of *Ochradenus baccatus* microshoots declined with increasing sugar concentration in the media, but at the highest concentrations of 0.4M mannitol, the micorshoots could not survive. Generally, aqueous extracts of the stressed microshoots were more effective against the tested microbial strains than the methanolic extracts in most experiments. *Staphylococcus aureus* was found to be the most affected microbe to both extract types. Also, exposing the microshoots to osmotic stress had improved antimicrobial powers in both extracts types. Aqueous extract of microshoots that pre-grew in media with (0.4 M sucrose) was interestingly found to inhibit growth of *Staphylococcus aureus* and *Candida albicans* with minimal inhibitory concentration (MIC) values of (0.195, 0.78 mg/ml). These values were similar to those obtained from the antibiotic treatments. Other biotechnological techniques like genetic transformation are suggested to be also used for production of elite strains of *Ochradenus baccatus* with super antimicrobial potential.

Keywords: Antimicrobial activity; *in vitro*; microshoots; *Ochradenus baccatus*; Osmotic agent, MIC, mannitol, sucrose.

1. INTRODUCTION

For ages, plants comprised a source for defense and prevention of disease [1-6]. Interestingly, millions of people consider plant medicine as the main source of health care[7]. Recently, synthetic antibiotics were reported to have a close relation to the high mortality rates among people due to the harmful outcomes of overuse and misuse of antimicrobial treatments on vital body organs and cells, as well as adverse effect on the immune system [8-10].

To find a solution there is a need to find alternatives to these chemical antibiotics. However, establishment of robust and suitable methods to provide continuous and effective alternatives against microbes might be provided

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Received: 29/3/2022 Accepted:22/7/2022.
DOI: https://doi.org/10.35516/jjps.v16i1.1066

by tissue culture techniques, which are independent on external environmental conditions [11-14]. *Ochradenus baccatus* Delile is a member of *Reseadasea* family [15] and widely spread in Middle Eastern deserts from Pakistan to the north of Africa [16]. In Jordan, *O. Baccatus* presents in Jordan valley, Dead Sea area, Wadi Araba, Al Karak and Wadi Mosa. This herb was reported to possess antibacterial, hepatoprotective, anti-inflammatory, anticancer, and antiviral activities which refer to the presence of several natural compounds such, as phenols and flavonoids [17]. *O. baccatus* is extensively used by the locals as a treatment of inflammations, high blood cholesterol and sexual disorders [18-19]. Unfortunately, uncontrolled collection of *Ochradenus baccatus* Delilah, is exposing this valuable herb to extinction. Finding a method that enables rapid and massive production of *Ochradenus baccatus* Delile with improved antimicrobial powers would contribute highly to rescue this plant degradation, and help to obtain a sustainable source of microshoots with elite curing powers without jeopardizing the plants grown in the wild.

Tissue culture techniques offer chances for production of effective compounds from the plant without depending on the wild [20], by applying techniques that allow rapid and massive production of microshoots which would consequently supply the target natural compounds *in vitro* [21]. This study was conducted to investigate the effect of osmotic stress on antimicrobial powers of two extract types collected from *Ochradenus baccatus in vitro* grown microshoots against different microbes, and to compare the results with those obtained from the wild type plant and the antibiotic.

2. MATERIALS AND METHODS:

2.1 *In vitro* establishment of mother stock of *Ochradenus baccatus*

In the materials section; all chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as mentioned specifically. Seeds of *Ochradenus baccatus* were sterilized by washing under tap water for 20 minutes then seeds were immersed in 4.0% sodium hypochlorite plus two drops of Tween–20 for 15 minutes. After that, the seeds were rinsed with autoclaved distilled water three times, then the seeds were soaked in 70% (v/v) ethanol solution for 30 seconds before being rinsed with sterile distilled [22].

The sterilized seeds were inoculated on the surface of petri dishes containing either of MS (1962) [23] solid media supplemented with 2.0 mg/L Gibberellic acid (GA_3), or hormone free MS media. Then the plates were kept at 21± 2°C under two light regimes consisted of complete darkness, or daily light regime in growth room conditions; of 16-h (photosynthetic photon flux density (PPFD) = 40 µmol. m^{-2} sec^{-1}) for two weeks. The germinated parts that developed cotyledonary stage were subcultured into 250 ml Erlenmeyer flasks (100 ml media / flask) containing hormone-free MS medium under daily light regime growth room conditions for four weeks.

After one month of seed germination nodal segments (1.0 cm) were subcultured into MS media supplemented with 1.0 mg/L of benzyl adenine (BA) [24] under completely sterile conditions. Cultures were incubated in growth room under daily light regime of 16-h (photosynthetic photon flux density (PPFD) = 40 µmol. m-2 sec-1) light, 8-h dark at 24±1°C. Microshoots were subcultured every four weeks and kept under the same mentioned conditions to initiate enough microshoots.

2.2 Studying the effect of different types and concentrations of osmotic agents on microshoots growth of *Ochradenus baccatus*

Microshoots were subcultured onto MS medium without growth regulators for 3-4 days to eliminate the carry-over effects of growth regulator. Then, 1.0 cm length of *Ochradenus baccatus* microshoot were subcultured into half strength (HS) MS medium supplemented with (0.1, 0.2, 0.3 or 0.4 M) of either sucrose, sorbitol or mannitol of 20 replicates of each treatment, the data were collected after 8 weeks of culture for microshoots length and number of microshoots, then samples were dried and kept till use for extraction.

2.3 Assessment of antimicrobial activity of wild and *in vitro* grown microshoots

2.3.1 Plant extracts preparation

Samples of wild type and in *vitro* grown microshoots of *Ochradenus baccatus* were dried in the shade for two weeks, and then grounded into fine powder by using a blender. The wild type samples (shoots and leaves) were collected from 31° 59 6 223 N, 35° 59 00 73 E (AL-Zarah) Dead Sea area, May 2016.

For aqueous extract, 8.0 g of the dried plant powder were taken from samples of wild type in addition to the in vitro grown microshoots (grown either under stressing or normal in vitro growth conditions) and were mixed with 50 ml autoclaved distilled water in clean bottles for 24 h at 24°C with shaking using incubator shaker (Sheldon Manufacturing, Inc.®; Cornelius, OR USA), then centrifuged at 2000 rpm for 10 min, before the resulted supernatant was collected [25]. After that, the water was evaporated by a rotary vacuum evaporator (RE300 rotary evaporator; Stuart Vacuum Pump - RE3022C, Staffordshire, UK); [26]. The residue was weighed, then dissolved in dimethyl sulfoxide (DMSO) [27] to obtain 100 mg/ml of stock extract then stored in refrigerator.

Methanolic extract was prepared by stirring 1 g of dried plant powder of the wild plant, *in vitro* grown microshoots (osmotic stress, non- stressed) in 10 ml of 100% methanol for 4h [25], followed by centrifugation at 2000 rpm for 10 minutes. After evaporating the methanol by rotary vacuum evaporator, the supernatant was dissolved and stored in DMSO at a concentration of 100 mg/ml as stock extract and stored in refrigerator [26]. Extracts from all these preparations were tested for purity by plotting them on Mueller Hinton Agar (MHA) and incubated for 24 hours at 37°C.

2.3.2. Evaluating antimicrobial activity

Five pathogenic microbes were selected for this study. Of these microbes are Gram-negative bacteria (e.g., *Escherichia coli* ATCC (8739) and *Klebsiella pneumonia* ATCC (31488)) and Gram-positive bacteria (e.g., *Staphylococcus aureus* ATCC (6538) and *Bacillus subtilis* ATCC (6633)), and one species of Fungi called *Candida albicans* purchased from ATCC (10231). These strains were supplemented by the microbiology laboratory at Hamdi Mango Center for Scientific Research.

2.3.2.1 *Inoculum Standardization*

Bacterial – fungal aliquots were prepared by dissolving one well - isolated bacterial colony into 3 ml of 0.9 % NaCl, while isolated fungi into 3 ml of sterilized distilled water. Then, the turbidity adjusted to 0.5 McFarland standard, to obtain aliquots containing approximately 1x10^8 CFU/ml.

2.3.2.2 *Bacterial and fungal broth Preparation*

Bacterial and fungal broth at 1:100 dilution was prepared by transferring 10 µl of previous prepared aliquots to 1.0 ml of Muller Hinton broth for bacteria, and to 1.0 ml of potato dextrose broth for fungi in order to carry out antimicrobial assay.

2.3.2.3 *Antimicrobial assay (Broth Microdilution)*

Different *O. baccatus* extracts; wild and *in vitro* grown microshoots (osmotic stress, non-stressed) were used to study their antimicrobial activity. MIC was determined by broth microdilution method, using 96-wells microplates. To do this, 50µl of Muller Hinton broth were added to each well of the first row (well A1) then serially diluted from (A1 to A10) by taking 50 µl of each well on the same row making a two-fold serial dilution ranging from concentration 25 to 0.09 mg/ml of extract, the last 50µl were discarded. Then, the ten wells were inoculated with 50µl of previously prepared bacterial or fungal broth.

Well number eleven in each row consisted of nutrient broth plus Tetracycline (10 mg/mL) and was used as a control. Testing50µl of DMSO instead of the extract was carried out to confirm the lack of interference of DMSO. The plates were covered and incubated at 37ºC for 24 hr. After the incubation period, the plates were scanned with an ELISA reader (BioTek® 800™ TS Absorbance Reader instrument, USA) at 600 nm [27] for bacteria, and at 405 nm for fungi [28]. The lowest concentration of extract that prevent microbial growth represented MIC [29]. Each

experiment in section 2.3 (Assessment of antimicrobial activity of wild and in vitro grown microshoots) was repeated three times with three replications.

2.4 Experimental design

Treatments of the experiment: (Effect of different types and levels of osmotic agents on microshoots growth of *Ochradenus baccatus*) were arranged in a completely randomized design (CRD). Each treatment consisted of twenty replicates (test tube) each with one microshoot to find out the effect of osmotic agent type and level on growth parameters of the microshoots (microshoot length and number of new proliferated shoots). The collected data were statistically analyzed using SPSS analysis system. Analysis of variance (ANOVA) was used and means separation was performed at probability level of 0.05 according to the Tukey's HSD. Antimicrobial experiments were repeated three times, and as mean values collected from antimicrobial experiments were discrete values (observed (+), not observed (-) they didn't undergo statistical analysis.

3.0 RESULTS AND DISCUSSION

3.1 Effects of different osmotic agents on *in vitro* growth of microshoots

Growth responses of *O. baccatus* microshoots varied according to the osmotic agent type and concentrations. Sucrose at the concentration of 0.1 M (the control) was obviously the best one for the growth of *O. baccatus* microshoots, as shoot length reached (5.4 cm) and 4.5 new microshoots developed (as seen in Table 1). However, increasing sucrose concentrations resulted in a clear reduction in the growth parameters to reach the minimum level at 0.4 M concentration of sucrose (as found in Table 1 and Figure 1). Our data were in agreement with results reported by studies [30- 32] who found that the growth and proliferation of *Achilliae fragrantissima, Thymbra spicata* and *Teucrium polium* microshoots decreased when grown under similar concentrations of sucrose. Similar trend of reduction in the growth parameters of microshoots when they exposed to similar range of concentrations of sorbitol and mannitol. However, the growth reduction was more severe in microshoots grown in 0.4 M concentration of mannitol comparing to other concentrations and other types of sugars with no survival microshoots.(Table 1). The continuous exposure of mannitol was reported to be toxic to the plant cells of *Oryza sativa* L. and *Ruta graveolens*. High concentration of mannitol decreased water uptake, decreased cell division, increased electrolyte leakage in the microshoots [33-34]. It is commonly known that adding high levels of sugars to the culture media would restrict water availability to plant cells and expose them to osmotic stress, which would force the cells to minimize their division as a defense mechanism [35].

Table 1: Effects of osmotic agent type and level on *in vitro* growth of *Ochradenus baccatus* microshoots after incubation for (8 weeks)

Concentration (M)	**Microshoots length (cm)**		
	Sucrose	**Sorbitol**	**Mannitol**
0.1 (control)	5.4 ± 0.31	4.1 ± 0.05	3.3 ± 0.07
0.2	4.1 ± 0.15	3.5 ± 0.05	2.2 ± 0.10
0.3	2.3 ± 0.02	1.7 ± 0.04	1.2 ± 0.07
0.4	1.1 ± 0.01	1.4 ± 0.05	-
	Number of proliferated shoots		
	Sorbitol	**Sucrose**	**Mannitol**
0.1	3.3 ± 0.41	4.5 ± 0.05	2.16 ± 0.07
0.2	2.1 ± 0.15	3.6 ± 0.25	1.2 ± 0.13

Concentration (M)	Microshoots length (cm)		
0.3	1.3 ± 0.02	2.8 ± 0.14	1.0 ± 0.07
0.4	1.1 ± 0.08	2.1 ± 0.05	-

(-): microshoots died after 8 weeks of incubation in this treatment

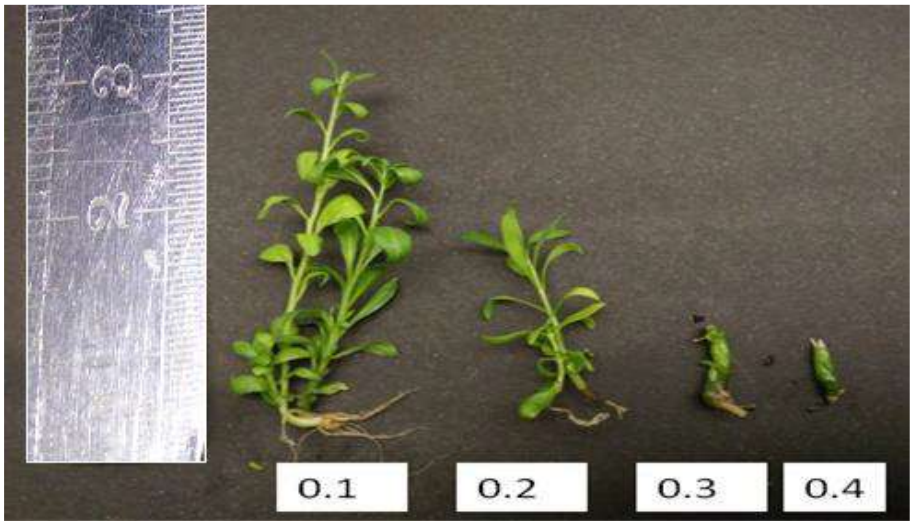

Figure 1: Microshoots length on MS media supplemented with (0.1, 0.2, 0.3, 0.4) M sucrose after 8 weeks.

3.2 Assessment of antimicrobial activity

3.2.1 ***Antimicrobial activity of in vitro grown Ochradenus baccatus microshoots under different concentrations of sucrose***

Antimicrobial activity was detected in both methanolic and aqueous extracts of *Ochradenus baccatus*. Interestingly, extracts collected from the microshoots pregrown in growth media contained increased sucrose levels have shown increasing antimicrobial powers of both extracts, although growth parameters of microshoot were decreased (Tables 1, 2). For example, antimicrobial potential against *Staphylococcus aureus* and *Candida albicans* was highly improved in methanolic extracts of *Ochradenus baccatus* microshoots grown in media with (0.4 M sucrose) with MIC values of 0.39 and 3.12 mg/ml, respectively. These MIC were similar to results obtained from the wild-type plant and the antibiotic with MIC values of 0.195, 0.78 mg/ m) (Table 2).

Adding high levels of sucrose to the culture media was reported to improve the production of secondary metabolites in cell and organ cultures for many plant species [36-39] and consequently improve antimicrobial powers of the resulted extract although growth of microshoots was adversely affected [40]. For example, cell culture of *Ginkgo biloba* showed maximum growth at 3% sucrose, but significant decline in the cell biomass at higher levels of sucrose of 5 and 7%. Interestingly, the aforementioned concentrations were the best for production of active ingredients (such as ginkgolides and bilobalides) [40].

Moreover, our data revealed that *Staphylococcus aureus* was the most affected microbe to both extract types of the microshoots., it was clear from the results that aqueous extract of the microshoots and the wild-type plant had stronger inhibition powers against all tested microbes compared to methanolic extracts (Table 2). This indicated that antimicrobial powers of the microshoots of *O. baccatus* varied with sucrose concentrations and extract type. Our findings were comparable with that obtained by [25] study who

found, that activity of *O. baccatus* aqueous extracts against nematode were more effective than methanolic extracts. It is worth mentioning that antimicrobial properties of *O. baccatus* were attributed to active ingredients produced after glucosinolate hydrolysis by enzyme myrosinase. The hydrolysis only happens in the presence of water and moderate temperature [41], which might explain the superiority of aqueous extracts over methanolic extracts.

On the other hand, study [19] found that other extract types (ethanolic and n-hexane extracts) of *O. baccatus* wild type plants were more efficient than aqueous and methanolic extracts at killing microbes, as ethanolic extract was effective against *Staphylococcus aureus; Escherichia coli*; and the fungus, *Candida albicans*, whereas n-hexane extracts was effective against *Candida albicans.*

Table 2. Effect of different concentrations of sucrose on antimicrobial activity of methanolic and aqueous extracts collected from *in vitro* grown *Ochradenus baccatus* microshoots growing under osmotic stressing conditions

Methanolic extract						
Sucrose concentration (M)						
Treatment	**0.1**	**0.2**	**0.3**	**0.4**	**Wild-type plant**	**Tetracycline (control)**
Staphylococcus aureus	3.12	1.56	0.78	0.39	0.39	0.195
E. coli	-	6.24	6.24	3.12	3.12	0.39
Klebsiella pneumoniae	6.24	6.24	3.12	3.12	1.56	0.78
Bacillus subtilis	3.12	3.12	1.56	1.56	0.78	0.195
Candida albicans	6.24	6.24	3.12	3.12	3.12	0.78
Aqueous extract						
Sucrose concentration (M)						
Treatment	0.1	0.2	0.3	0.4	Wild type plant	Tetracycline (control)
Staphylococcus aureus	1.56	0.78	0.39	0.195	0.195	0.195
E. coli	6.24	3.12	1.56	1.56	0.78	0.39
Klebsiella pneumoniae	6.24	6.24	3.12	1.56	0.78	0.78
Bacillus subtilis	1.56	1.56	1.56	0.78	0.39	0.195
Candida albicans	3.12	3.12	1.56	0.78	0.78	0.78

* Values represent the means of minimal inhibitory concentrations MIC (mg/ml) of the extract needed to inhibit growth of each microbe. (-): No inhibition observed.

3.2.2 ***Antimicrobial activity of in vitro grown Ochradenus baccatus microshoots under different concentrations of sorbitol***

Antimicrobial powers of the microshoots increased in microshoots pregrwon in MS media supplemented with higher concentrations of sorbitol levels (Table 3). Both extract types from the microshoots showed antimicrobial activities against most tested microbes except *Candida albicans* (Table 3). On the other hand, growth of *Candida albicans* was inhibited upon treatment with aqueous and methanolic extracts of the wild-type plant with MIC values of 3.12 and 0.78 mg/ml, respectively (Table 3). *Staphylococcus aureus* was again the most affected microbe when exposed to sorbitol treatment in both extract types (Table 3). Additionally, aqueous extract kept showing better performance against all microbes than methanolic extract (Table 3).

Table 3. Effect of different concentrations of sorbitol on antimicrobial activity of methanolic and aqueous extracts collected from in vitro grown *Ochradenus baccatus* microshoots growing under osmotic stressing conditions

Methanolic extract						
Sorbitol concentration (M)						
Treatment	**0.1**	**0.2**	**0.3**	**0.4**	**Wild type plant**	**Tetracycline (control)**
Staphylococcus aureus	3.12	1.56	0.78	0.78	0.39	0.195
E. coli	-	-	12.48	6.24	3.12	0.39
Klebsiella pneumoniae	12.48	6.24	3.12	3.12	1.56	0.78
Bacillus subtilis	3.12	3.12	1.56	1.56	0.78	0.195
Candida albicans	-	-	-	-	3.12	0.78
Aqueous extract						
Sorbitol concentration (M)						
Treatment	0.1	0.2	0.3	0.4	Wild type plant	Tetracycline (control)
Staphylococcus aureus	3.12	1.56	0.39	0.39	0.195	0.195
E. coli	-	6.24	3.12	3.12	0.78	0.39
Klebsiella pneumoniae	-	6.24	1.56	1.56	0.78	0.78
Bacillus subtilis	3.12	3.12	1.56	0.78	0.39	0.195
Candida albicans	-	-	-	-	0.78	0.78

* Values represent the means of minimal inhibitory concentrations MIC (mg/ml) of the extract needed to inhibit growth of each microbe. (-): No inhibition observed.

3.2.3 ***Antimicrobial activity of in vitro grown Ochradenus baccatus microshoots under different concentrations of mannitol***

Aqueous and methanolic extracts of *Ochradenus baccatus* microshoots were observed to have antimicrobial action against three types of bacteria including *Staphylococcus aureus, Klebsiella pneumoniae* and *Bacillus subtilis upon exposure to mannitol (*as seen in Table 4*).* This was exclusively noticed in extracts collected from microshoots pregrown with increasing concentrations of mannitol, with the exception for the highest concentration of 0.4M mannitol because microshoots could not survive after 8 weeks of incubation at this concentration (Table 4). Also, it was clear from Table 4 that growth of *E. coli* was inhibited when exposed to aqueous and methanolic extract of the wild-type plant with MIC of (12.48 and 3.12 mg/ml, respectively), but it wasn't affected by any extract type collected from the microshoots. Meanwhile, *Candida albicans* wasn't affected by any extract type of *O. baccatus* (as seen in Table 4).

Many stress physiology research articles have shown that adding high concentrations of sugars was found to improve secondary metabolites production in *in vitro* grown plants [37-38; 42]. This might explain that improvement in antimicrobial activity of both extracts of microshoots in response to the addition of high sugar concentrations.

Table 4. Effect of different concentrations of mannitol on antimicrobial activity of methanolic and aqueous extracts collected from in vitro grown *Ochradenus baccatus* microshoots growing under osmotic stressing conditions

Methanolic extract					
Mannitol concentration (M)					
Treatment	0.1	0.2	0.3	Wild type plant	Tetracycline (control)
Staphylococcus aureus	3.12	3.12	1.56	0.39	0.195
E. coli	-	-	-	12.48	0.39
Klebsiella pneumoniae	6.24	6.24	3.12	1.56	0.78
Bacillus subtilis	6.24	6.24	1.56	0.78	0.195
Candida albicans	-	-	-	-	0.78
Aqueous extract					
Mannitol concentration (M)					
Treatment	0.1	0.2	0.3	Wild type plant	Tetracycline (control)
Staphylococcus aureus	3.12	1.56	0.39	0.195	0.195
E. coli	-	-	-	3.12	0.39
Klebsiella pneumoniae	6.24	6.24	3.12	0.78	0.78
Bacillus subtilis	3.12	1.56	1.56	0.39	0.195
Candida albicans	-	-	-	-	0.78

* Values represent the means of minimal inhibitory concentrations MIC (mg/ml) of the extract needed to inhibit growth of each microbe. (-): No inhibition observed.

4. CONCLUSIONS

It can be concluded from the obtained data that growth responses of *Ochradenus baccatus* microshoots varied with osmotic agent type and level. Adding 0.1M sucrose to the media resulted in the best growth for the microshoots, while adding higher levels of all sugar types affected microshoots growth adversely.

Meanwhile, antimicrobial activity of *Ochradenus baccatus* was found to be enhanced in extracts collected from microshoots pregrown under osmostressing conditions. The reason behind this can be due to fact that plant cells tend to produce more active ingredients including secondary metabolites to balance cell water potential in order to reduce water loss resulted from cells exposure to high osmotic stressing conditions. However, our results showed that the best antimicrobial results were recorded by extracts collected from microshoots samples pregrown in vitro in media contained either (0.3 or 0.4) M of sucrose or sorbitol.

More research is needed on the extract of *Ochradenus baccatus* to find out exactly the types of secondary metabolite that are responsible for the antimicrobial powers of this plant and how other tissue culture techniques can be applied to improve production of these compounds.

5. Acknowledgement

Authors would like to thank the University of Jordan, The Deanship of Academic Research, for funding this research project. Also, many thanks are extended to all members of Hamdi Mango Center for Scientific Research for their generous help and support.

6. Conflict Of Interest:

The authors can hereby confirm that they have no conflict of interest in this manuscript.

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الإجهاد الاسموزي يعزز النشاط المضاد للميكروبات لنبات الأرضه *Ochradenus Baccatus* Delile المزروع داخل الانابيب في المختبر ضد ميكروبات مختاره

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ملخص

نبات الارضة *Ochradenus baccatus* Delile هو نبات طبي بري ينتج العديد من المركبات الطبيعية ذات الفوائد الطبية. في هذه الدراسة، تم تعريض الفروع الصغيرة من نبات الارضه *Ochradenus baccatus* داخل الانابيب الى ظروف الإجهاد الاسموزي و التي تتكون من وسائط غذائية صلبة موراشيج و سكوج Murashige و Skoog (MS) و التي تحتوي على عوامل أسموزية مختلفة (أنواع سكريات) في مدى من التركيزات (بما في ذلك 0.1 ، 0.2 ، 0.3 أو 0.4 مولار) . كان الهدف هو اختبار تأثير هذه السكريات على نمو السيقان الدقيقة للنبات داخل الانابيب والأنشطة المضادة للميكروبات لهذا النبات ضد سلالات مختارة من البكتيريا وسلالة واحدة من الفطريات. لقد وجدنا أن عوامل النمو (بما في ذلك طول النبتة وتكاثرها) من سيقان نبات الارضه داخل الانابيب *Ochradenus baccatus* انخفضت مع زيادة تركيز السكر في الوسط الغذائي ، ولكن عند أعلى تركيز 0.4 مولار مانيتول، لم تتمكن السويقات الدقيقة داخل الانابيب من البقاء. بشكل عام ، كانت المستخلصات المائية للسويقات الدقيقة للنبات داخل الانابيب المعرضه للإجهاد الاسموزي أكثر فعالية ضد السلالات الميكروبية المختبرة من المستخلصات الميثانولية في معظم التجارب. تم العثور على المكورات العنقودية الذهبية *Staphylococcus aureus* لتكون الميكروب الأكثر تضررا من كلا النوعين من المستخلصات. أيضًا ، أدى تعريض البراعم الدقيقة للإجهاد الاسموزي إلى تحسين القوى المضادة للميكروبات في كلا النوعين من المستخلصات. المثير للاهتمام أن المستخلص المائي السيقان الدقيقة للنبات داخل الانابيب التي نمت مسبقًا في الوسط الغذائي باستخدام (0.4 مولار سكروز) عملت على تثبيط نمو المكورات العنقودية الذهبية *Staphylococcus aureus* والخمائر البيضاء *Candida albicans* باستخدام طريقة التثبيط عند التركيز الاقل (MIC) بقيم (0.195 ، 0.78 مغم / مل). كانت هذه القيم مماثلة لتلك التي تم الحصول عليها من العلاجات بالمضادات الحيوية. يُقترح أيضًا استخدام تقنيات التكنولوجيا الحيوية الأخرى مثل التحول الجيني لإنتاج سلالات النخبة من *Ochradenus baccatus* مع إمكانات فائقة لمضادات الميكروبات.

الكلمات الدالة: نشاط مضاد للميكروبات، داخل الانابيب، السويقات، نبات الارضه، عامل أسموزي، التثبيط عند التركيز الاقل (MIC)، سكر مانيتول، سكر سكروز.

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تاريخ استلام البحث 2022/3/29 وتاريخ قبوله للنشر 2022/7/22.