Antimicrobial efficacy and cryogenic conservation of *Solanum villosum* grown under in vitro environment

Rida A. Shibli (✉ r.shibli@ju.edu.jo)

University of Jordan

Tamara Al-Qudah

UJ: The University of Jordan

Ahmad Zatimeh

Al-Balqa' Applied University

Reham Tahtamouni

Al-Balqa' Applied University

Saida Abu Mallouh

UJ: The University of Jordan

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Abstract

Solanum villosum is a wild distinguished medicinal herb that has few studies describing its medicinal properties with no sustainable production and conservation studies. In this study, in vitro cultures of microshoots and callus of *Solanum villosum* were multiplied in vitro on Murashige and Skoog media supplemented with different growth regulators. The MS media that have 2,4 Dichlorophenoxy acetic acid (2,4-D) and Kinetin at the levels of (2.0, 1.2 mg/L; respectively) were used for the induction and multiplication of callus that yielded the highest fresh weight of (6.03 ± 0.15) g. Cryopreservation was applied successfully to seeds using the dehydration technique and for both microshoots and callus using v-cryoplate technique. The highest regrowth rate was obtained in callus (95 ± 0.13) after pre-culturing callus on 0.3 M sucrose for 5 days. The most inhibited bacteria strains by microshoots extracts were, *Klebsiella pneumoniae* and *Staphylococcus epidermidis* (30 ± 0.25 and 25 ± 0.17 mm; respectively) using the disk diffusion assay. In MIC method; The extract obtained from microshoots achieved best results of MIC values (9.77 and 39.06 ($\mu\text{g/ml}$) against (*Micrococcus luteus* and *Klebsiella pneumonia*; respectively). A sustainable protocol of callus culture and cryopreservation protocol for in vitro *Solanum villosum* has been set for the first time which makes this research novel. Furthermore, promising results of antimicrobial activity for both in vitro and wild *Solanum villosum* were obtained.

1.0 Introduction

Solanum villosum is a wild medicinal herb that belongs to the family Solanaceae. The common name of *Solanum villosum* is red-fruit nightshade. It has a wide spread distribution in Jordan and the middle east regions (Tahtamouni et al., 2017). *Solanum villosum* has different uses in medicine and folk medicine. This because it has different chemical compounds such as alkaloids, flavonoids, saponins and many other (Amir and Kumar, 2004). *Solanum villosum* has many documented activities against microbes with many natural compounds. *Solanum villosum* has many different natural products which can be used in medicine, manufacturing (Balouri et al., 2016; Sridhar et al., 2011; Ochoa-Villarreal et al., 2016). Different microbe types have developed their resistance against many antibiotics and manufactured drugs. This opened the way to use different natural sources of chemicals, especially from plant sources. This in order to use as an alternative medicinal components in pharmaceutical sources. (Guschin et al., 2015, Martin et al., 2015; Iwu et al., 1999; Jaganthan et al., 2015). Besides that; Most of manufactured antibiotics may have different side effects which could be not safe or lethal to human besides their high costs. Recently, most of the research orientation has concentrated on the folk medicine and natural products as an alternative to antibiotics and different medicines (Benkeblia, 2004).

Furthermore, *Solanum villosum* could be used in food as edible or as spices, especially in India, also as tea herb in some counties in Africa (Venkatesh et al., 2014). Till now, little concerns have been directed to keep *Solanum villosum* as an important genetic resource for sustainable. Tissue culture is a potent tool that enhances massive plant propagation, callus culture, and cryopreservation under controlled conditions in a short time period (Tahtamouni et al., 2017, Shibli et al., 2016). Cryopreservation of *S. villosum* in Jordan is now an urgent need; this is due to the continuous degradation of their natural

populations and there are no conservation efforts had been taken (Taifour and El-Oqla, 2014). The *in vitro* culture and cryopreservation of wild medicinal plants can offer a permanent resource to study these important plants (Yamamoto et al. 2012; Niino et al. 2014). Besides that, it permits a better understanding of the mechanism by which plant cells synthesize secondary metabolites and how these metabolites would react against microbes (Balouri et al., 2016). In this study keeping genetic resources of *Solanum villosum* via callus culture and v-cryoplate cryopreservation technique was evaluated with such medicinal value of the *in vitro* culture. This study aimed to study the factors that affect on the *in vitro* conditions of *Solanum villosum* in order to propagate the callus and cryopreservation of the plant materials and seeds. Also study the potential of the activity of different plant extracts against different microbes. Furthermore, according to literature this is the first report addresses the conservation of this important medicinal plant and the *in vitro* plant material activity against microbial activities.

2.0 Materials And Methods

In vitro seedlings were first established from seeds. Seed collections and other information related to seed source are documented in Tahtamouni et al., (2017). The *in vitro* culture was done using media of Murashige and Skoog, 1962 (MS) without hormones. Then *in vitro* shoots had been multiplied on media supplemented with (1.5 mg/L) of Thidiazuron (TDZ) as documented previously by Tahtamouni et al., (2017).

2.1 Callus induction and Multiplication.

Callus was induced from leaf discs excised from the *in vitro* grown microshoots sub-cultured in Petri dishes containing solid MS media supplemented with (2.0 mg/L) of 2,4 Dichlorophenoxy acetic acid (2,4-D) in combination with (1.2 mg/L) of Kinetin plus 0.1 M sucrose. Induced calli (5 mm dia.) from leaves source was then transferred to MS media have the following levels (0.4, 1.2, 2.0 mg/L) of different cytokinin; Kinetin, BA or TDZ in addition to 2.0 mg/L 2,4-D as a constant with all levels used. The control media have only the 2,4-D growth regulator at the concentration of 2.0 mg/L. Each treatment has ten replicates with 2 pieces of callus segments. Data were collected after four weeks for callus diameter and fresh weight.

2.2 Cryopreservation of seeds:

The cryopreservation protocol of the seeds was applied according to Al-Abdallat et al., (2017). Seeds of the same genotype and collection information was documented by Tahtamouni et al., (2017). *Solanum villosum* seeds were then dried and stored at 24 °C in dark bags till use. A sample contains about 200 seeds were dried using silica gel to release the extra water from seeds and to adjust the relative humidity. This in order to achieve the equilibrium state of the seeds that has no difference between the initial seeds fresh weight and the final dry weight. Then the dried seeds were subjected to the cryopreservation process by putting the seeds in 2 ml cryovials.; each cryovial contains about twenty seeds; with ten replicates or cryovials. Then, the cryovials were plunged in the liquid nitrogen for more than 1 hour. Besides that, the control treatment was consisted of cryovials without merging in liquid nitrogen.

Thawing was applied by picking up cryovials from liquid nitrogen and immersing them directly in the water bath at the temperature (40 °C) for 20 min. Then seeds were taken outside cryovials after warming and tested for germination on moistened double-layer filter papers in Petri-dishes. About 5 seeds were cultured per Petridis and kept in an incubator at 24°C. Data were collected for seed germination at different intervals of 4 weeks as a percentage of germination.

2.3 Cryopreservation (v-cryoplate) of Calli and microshoots.

The cryopreservation protocol of the *Solanum villosum* in vitro explants of microshoots and calli was applied according to Al-Abdallat et al., (2017; d Yamamoto et al., (2012). The *Solanum villosum* in vitro explants of microshoots and calli were transferred into MS media without hormones supplemented with 0.3 M sucrose. After that, cultures were incubated for 1, 3, or 5 days under dark in the growth room conditions (22–25°C). Then the v-cryoplates which have about 10 wells per each were used for cryopreservation of callus or microshoots. This was done by attaching each explant on the well by using alginate gel. After that the calcium chloride CaCl_2 were used on wells in order to give polymerization on the explants attached to the wells inside v-cryoplate. Each experiment included different preculture periods and was replicated ten times. Each cryoplate is considered one replicate with 10 explants or ten wells.

Then the cryoplates were immersed in the loading solution which composed of (2 M glycerol + 1.0 M sucrose) for 20 min in order to do osmoprotection to the explants. On the other hand, the second process included dehydration of explants on the cryoplates for 20 min using the vitrification solution (PVS2) which composed of (HF-MS + 30% glycerol + 15% ethylene glycol (EG) + 15% dimethylsulfoxide (DMSO) and 0.4 M sucrose). After the dehydration process of the cryoplates which containing the explants, The cryoplates were immersed directly in the liquid nitrogen for more than 2 min. Then, the cryoplate were removed quickly to the warming solution which composed of unloading solution (1 M sucrose) at 25 °C. After warming of the explants, they were transferred to MS media have 0.1 M sucrose and placed in the growth room at 25 °C at dark conditions for one week. After that, the cultures were transferred to the ordinary conditions in the growth room (16/8 hr of (light/dark)) regime and $40\text{--}45\ \mu\text{molm}^{-2}\text{s}^{-1}$ light intensity). The explants were monitored for their regrowth after cryopreservation. The regrowth data were taken for the change in color, size or bud initiation in the explants. Data were recorded after four weeks. On the other hand, the data on the survival were taken by counting the number of Survived explants per total explants. The surviving explants were measured visually through the red color in the explants using the tetrazolium test (2,3,5-triphenyl tetrazolium chloride)

2.4 Antimicrobial activities:

The leaves of wild *Solanum villosum* in addition to in vitro shoots and in vitro callus were dried in an oven at 60°C, then grounded into fine powder. The extraction was carried out with methanol with a ratio of 1:10. Then the extracts were filtered and dried via the rotary evaporator. The dried samples, were then diluted by DMSO to 100mg/ml as a final concentration. *Solanum villosum* extracts after that were experimentd on differnt bacteria species (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC

6633, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 10240, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli*, *Salmonella sp.*, *Klebsiella pneumoniae* ATCC 31488, and *Erwinia carotovora*). Besides that, one fungi species of were used *Candida albicans*. ATCC 10231. The antimicrobial activity of *Solanum villosum* extract was determined using both disk diffusion assay and microdilution assay. The first method of Disc diffusion was experimted via making discs of 6 mm in the surface of Muller Hinton agar. Then about of 100 µl of the bactial suspension and 10 mg of the extract were loaded to disc (Karlsnose, 2010). Standard antibiotics (Tetracycline 10 mg/ml) were used as reference or positive control. Antibacterial activity was After that the zone of inhibition was recorded in the incubator conditions after one day at 37C°. The second method (MIC); Micro dilution test were experimented using dillution of extracts up to ten dilutions (5 mg to 9.77 µg) as mentioned in NCCLS (2000). The bacteria inoculate had about 105 CFU/ml of bacteria and the results were taken visually in the plate shaker incubator after one day at 37°C .

Statistical Analysis.

Treatments of all experiments were distributed using Complete Randomized Design (CRD) with ten replications and 2 explant/ replicate. Data recorded from each treatment were analyzed separately using (ANOVA); analysis of variance via (SPSS) software, version (19). The standard errors were calaculated and the seperation of means were done using Tukey's HSD test at 0.05 probability level.

3.0 Results

3.1 Callus multiplication

Different media with different growth regulators have been used for callus induction (data not shown). The media containing (2.0 mg/L 2,4-D) was used to induce callus as they gave healthy and friable white-yellowish callus segments. For callus multiplication, different growth regulators types and levels were used as shown in Table 1. The highest increase in callus fresh weight (6.03 ± 0.15 g) was obtained at 1.2 mg/L Kinetin as shown in Fig. 1. While, when the concentration of Kinetin was increased up to 2.0 mg/L, the callus fresh weight was decreased to (4.65 ± 0.032 g). Adding BA has shown a moderate impact on callus growth as we can see from Table 1. The maximum weight of callus (3.14 ± 0.16) was obtained using BA at 2.0 mg/L. A negative impact of TDZ was shown on callus texture, color, and weight. It has produced a compacted and a brownish callus culture.

Table 1

Callus cultures of *Solanum villosum* growth at different concentration of (BA, TDZ or Kin) with 2.0 mg/L 2,4-D .

Concentration mg/L	Fresh weight	Texture	Color
Kin			
^z Control	2.05 ± 0.16 c*	Friable	Yellow
0.4	2.01 ± 0.02 c	Friable	Yellowish white
1.2	6.03 ± 0.15 a	Friable	Yellowish white
2.0	4.65 ± 0.032 b	Friable	Yellowish white
BA			
Control	2.05 ± 0.16 c	Friable	Yellow
0.4	2.03 ± 0.019 c	Friable	white
1.2	2.43 ± 0.14 b	Friable	white
2.0	3.14 ± 0.16 a	Friable	white
TDZ			
Control	2.05 ± 0.16 a	Friable	Yellow
0.4	1.9 ± 0.03 b	Compacted	brown
1.2	2.01 ± 0.15 a	Compacted	brown
2.0	2.06 ± 0.21 a	Compacted	brown
^z Control: MS media with 2.0 mg/L 2,4-D. * Different letters indicate that there are significant differences between means at ($p < 0.05$) according to Tukey HSD for each growth regulator. Each growth regulator was statistically analyzed separately.			

3.2 V-cryoplate cryopreservation:

Different pretreatment durations (1, 3, 5, and 7) days were used with 0.3 M sucrose to show the effect of regrowth of cryopreserved micro shoots and callus of *Solanum villosum*. As we can see from Table 2, the regrowth percentage of cryopreserved micro shoots and callus segments was optimized to (80.00 ± 0.12 , 95 ± 0.13 ; respectively); when they were pre-cultured on 0.3 M sucrose for 5 days. Micro shoots gave lower regrowth and survival rate than the callus of *Solanum villosum*. When the preculture period was prolonged up to 7 days the regrowth and survival rates were decreased for both callus and microshoots. The V-cryoplate cryopreservation was applied according to ()

Table 2
The effect of the preculture period of regrowth and survival of *Solanum villosum* in vitro callus and microshoots via v-cryoplate technique.

Pretreatment duration (Day)	Regrowth %	Survival %
microshoots		
1	0.00 ± 0.00*	25%
3	50.0 ± 0.3	80%
5	80.00 ± 0.12	85%
7	65 ± 0.32	75%
Callus		
1	0.00 ± 0.00*	30%
3	60 ± 0.12	85%
5	95 ± 0.13	100%
7	80 ± 0.14	90%
*Values represent means ± standard error.		

3.3 Seed cryopreservation experiment

All seeds were able to germinate after cryopreservation as shown below in (Table 3). The results indicate that the liquid nitrogen did not affect the seed germination. The germination percentage of the cryopreserved seeds was (96 ± 4.03) after four weeks of incubation period. This percentage was very close to the non treated seeds with liquid nitrogen after the same period of incubation. This promising data will be used in the cryopreservation of seeds for unlimited time, which will enrich the genetic resources backup.

Table 3

The effect of cryopreservation on the germination percentages of *Solanum villosum* seeds versus non-cryopresrvd seeds in the period around four weeks.

Week	Germination of non- cryopreserved seeds ^y	Germination of cryopresvred seeds
1	45 ± 5.07 b*	44 ± 2.24 b
2	54 ± 4.05 b	50 ± 3.43 b
3	85 ± 3.49 a	72 ± 5.02 a
4	100 ± 0.01 a	96 ± 4.03 a
^y Control: treatment: non- cryopreserved seeds, *The value in each cell is the Mean ± SD (standard error) according to Tukey's HSD test. The different letters express the significant difference between means at (p < 0.05) for the germinated seeds.		

3.4 Antimicrobial activity

The *Solanum villosum* microshoots extracts were efficient in prohibition of the microbes, and these results were very similar to antibiotic and wild plant extracts results. For disk diffusion assay; the most inhibited bacteria strains were, *Klebsiella pneumoniae* and *Staphylococcus epidermidis* with a growth inhibition zone diameter of (30 ± 0.25, 25 ± 0.17 mm; respectively) using micro shoots extracts as shown in Table 4. These two bacterial types were more effective or their results were very close to the antibiotic results. In our study, callus extract was less effective in antibacterial activity than microshoots and resulted in maximum inhibition zones of (20 ± 0.32, 18.7 ± 0.28 mm); recorded in (*Klebsiella pneumoniae* and *Staphylococcus epidermidis*; respectively). For a *Solanum villosum* activity against *Candida*; the inhibition zone of *Candida albicans* by callus extract using disc diffusion assay was (10 mm) diameter and inhibition of growth zone was (20 mm) for wild plant extracts as shown in Table 4.

Table (4)

The inhibition zones (mm) obtained from different *Solanum villosum* explants extracts (callus, microshoots and wild plant) using the Disc diffusion assay. The (antibiotic) is positive control.

Bacterial strain	<i>Solanum villosum</i>			
	Inhibition zone diameter (mm)			
	Wild	Microshoots	In vitro callus	Tetracycline
<i>Bacillus subtilis</i> ATCC 6633	10 ± 0.24 b ^z	11.5 ± 0.06 b	N.D. ^y	21.60 ± 0.41 a
<i>Staphylococcus aureus</i> ATCC 25923	15 ± 0.21 a	12.3 ± 0.25 b	16.3 ± 0.15 a	15.7 ± 0.17 a
<i>Micrococcus luteus</i> ATCC 10240	20 ± 0.13	22.7 ± 0.32 b	14 ± 0.09 c	33.3 ± 0.13 a
<i>Staphylococcus epidermidis</i> ATCC 12228	22 ± 0.06 ab	25 ± 0.17 a	18.7 ± 0.28 b	14.7 ± 0.41 c
<i>Enterobacter aerogenes</i> ATCC 13048	12.5 ± 0.17 c	14 ± 0.09 b	11.3 ± 0.08 d	16.3 ± 0.04 a
<i>Klebsiella pneumoniae</i> ATCC 31488	20 ± 0.10 b	30 ± 0.25 a	20 ± 0.32 b	31.7 ± 0.21 a
<i>E. coli</i>	10 ± 0.42 c	12.3 ± 0.25 b	14 ± 0.42 a	10.7 ± 0.07 c
<i>Salmonella sp.</i>	10 ± 0.05 c	19.3 ± 0.25 b	21 ± 0.12 a	25.7 ± 0.23 a
<i>Erwinia carotovora</i>	8 ± 0.071 c	9 ± 0.25 c	11.3 ± 0.27b	15.3 ± 0.21 a
<i>Candida albicans</i> ATCC 10231	N.D.*	10.5 ± 0.07 b	10.3 ± 0.13 b	12 ± 0.07 a
^z Means ± standard error; different letters of means are separated according to Tukey HSD at P < 0.05 and have a significant difference. Each bacterial strain in each row was analyzed separately. ^y N.D : not detected.				

In MIC method; micro shoots extract was effective at MIC values of (9.77) µg/ml against *Micrococcus luteus*. Furthermore, *Klebsiella pneumoniae* and *Bacillus subtilis*) gave the same results of MIC (39.06 µg/ml) when the microshoots extracts was used as shown in the Table 5. On the other hand, callus and microshoots extracts achived higher prohibition results against *Candida albicans* compared with the wild plant extracts as shown in Table 5. The MIC value of *Candida albicans* by callus extract and wild plants was 9.7 (µg/ml) for both and was not detected in wild plant extarct.

Table (5)

The (MIC) minimum inhibitory concentration (μg) against different microbes using different *Solanum villosum* extracts.

Bacterial strain	<i>Solanum villosum</i>		
	Wild	microshoots	In vitro callus
	MIC (μg)		
<i>Bacillus subtilis</i> ATCC 6633	78.1	39.06	625
<i>Staphylococcus aureus</i> ATCC 25923	312.5	625	1250
<i>Micrococcus luteus</i> ATCC 10240	312.5	9.77	156.25
<i>Staphylococcus epidermidis</i> ATCC 12228	156.25	312.5	1250
<i>Enterobacter aerogenes</i> ATCC 13048	625	625	625
<i>Klebsiella pneumoniae</i> ATCC 31488	78.1	39.06	1250
<i>E. coli</i>	312.5	312.5	N.D
<i>Salmonella sp.</i>	78.1	312.5	625
<i>Erwinia carotovora</i>	625	312.5	625
<i>Candida albicans</i> ATCC 10231	N.D.*	9.77	9.77
*N.D : not detected			

4.0 Discussion

4.1 Callus multiplications: The callus of the *Solanum villosum* was successfully induced and multiplied in vitro using different plant growth regulators of cytokinins with 2,4-D as a constant supplement to the media. In our study, the most important growth regulator has played an important role in the callus induction was the Kinetin. Different studies had shown a positive impact of Kinetin on the callus culture when used in combination with 2,4-D. For example; callus cultures had been successfully produced using

Kinetin and 2,4-D in *Securinega suffruticosa* (Raj et al. 2015), *Viola uliginosa* (Slazak et al. 2015), and *Lantana camara* Linn (Tahtamouni et al., 2020). This may be due to that Kinetin and 2,4-D has a major role in the plant tissue un-differentiation process (callus). On the other hand, our results using TDZ were not promising for callus multiplication. This may be that TDZ did not work well for producing friable callus in *Solanum villosum* and some herbaceous shrubs. On the contrary; *Peganum harmala* L. callus cultures were maximized at (1.5 mg/L) TDZ with 2.0 mg/L 2, 4-D) and gave (2.88 g) of callus fresh weight (Zatimeh et al., 2017). In this study, BA showed a moderate effect on callus culture. The callus remained friable and white in color, but the fresh weight reached up to 3.14 ± 0.16 at 2.0 mg/L of BA. In contrast, Veraplakorn (2016) found that BA was the best for callus multiplication in *Lantana camara* L. when used with NAA. We can notice from these results, that the plant differentiation process depends on both the type of the plant bgrwth regulator and the plant species. From previous studies, we can say that each plant species has its own growth pathway.

4.2 V-cryoplate cryopreservation: In our study *Solanum villosum* microshoots and callus were successfully preserved in liquid nitrogen using the V - cryoplate technique. The pre culture period of the plant material also affected significantly the preservation process. Previous studies reported that the preculture period could increase the survival rate of cryopreserved plant materials (Al abdallat et al., 2017; Shibli et al., 2016). V- cryoplate was successfully used to cryopreserve *Foeniculum vulgar* (Shibli et al., 2016). Also, *Dianthus caryophyllus* L. were successfully cryopreserved via V- cryoplate (Sekizawa, et al., 2011). The cryopresrved germplasm of the mint had shown a full regrowth when the v-Cryo plate method was used (Yamamoto, et al., 2012). Besides that, The mat rush (*Juncus decipiens* Nakai) lateral buds we cryopresrved successfully by v-cryoplate technique and the regrowth percentage reached 88% (Niino, et al. 2014). From previous studies, we had noticed that v-cryoplate technique had increased the success percentage of the cryopreservation process for different plant species rather than other ordinary methods such as vitrification and dehydration methods.

4.3 Seed cryopreservation experiment: *Solanum villosum* seeds cryopreservation was very efficient method. This because the viability and germination percentage is affected less by liquid nitrogen. Different plant species were tested for seed cryopreservation with different germination percentages. For example, *Encholirium pedicellatum* cryopreserved seeds showed high germination after cryopreservation (Tarré et al., 2007). Also (*Solanum lycopersicum* L.) cryopreserved seeds showed a high percentage of germination after cryopreservation (Al-Abdallat et al., 2017). So the seed cryopresrvation method could be used as a viable and quick method for the genetic resources preservation issue.

4.4 Antimicrobial activity: In this study, *Solanum villosum* methanol extracts have shown a wide range of antimicrobial activity against different microbes used. The methanol extract has been reported for it is activity against different bacterial strains (Al-Qudah, 2020). Different extract types were used in other plant species against microbes. For example; Tahtamouni (2018) found that *Klebsiella pneumoniae* inhibition zone (13.0 mm) was highly inhibited using *Schinus molle* leaves ethanolic extracts. On the other hand, *Solanum nigrum* ethyl acetate seed extracts were very efficient in prohibition of *Pseudomonas*, *Proteous vulgaris*, *Klebsiella* with the zones of inhibition (20.5–21.0 mm) (Sridhar et al.,

2011). In our study the disk diffusion method was highly effected against *Klebsiella pneumoniae* and *Staphylococcus epidermidis* using the in vitro microshoots extracts. Two different methods of Agar disc diffusion and agar tube dilution were used in *Solanum villosum* to show the antimicrobial activity of their extracts (Abdel Gawwad et al., 2020). Abdel Gawwad et al., (2020) had used two extracts types (methanol and chloroform) and their activity reached up to 75% of prohibition against different bacteria species used. On the other hand, these two extracts gave about 50% of prohibition against fungi species used. Our results for the in vitro microshoots and callus extracts against microbes were recorded for the first time in *Solanum villosum*.

Furthermore the MIC method also was very promising using the extracts of the plant tissue culture materials of *Solanum villosum* against different used microbes. Previous studies had displayed different MIC effects from plant extracts on microbe's species. Tahtamouni (2018) found that the fruit or leaf ethanol extracts of *Schinus molle* were very effective in prohibition of *Micrococcus luteus* at the level (6.25 mg/ml) using a microdilution assay method. On the other hand, the methanol extracts from different plant parts of *Schinus mole* did not express an inhibition effect against *Micrococcus luteus* (Tahtamouni, 2018). *Solanum nigrum* extracts also were effective using the MIC method against different microbes mainly; *Pseudomonas putrida*, *Proteus vulgaris*, and *Klebsiella pneumonia* (Sridhar et al., 2011). Also, methanolic extracts of *S. torvum* roots had exhibited promising results and antibacterial and antifungal effects on all organisms tested in comparison with that observed in the leaves, stems, and inflorescence extracts (Bari et al., 2010). From previous studies, we can see that the plant species and the plant material source have the major effect on antimicrobial activity. Also, we had noticed that for some microbes the plant tissue culture extracts were very promising more than wild types. This result can be used in future to depend on the in vitro sources of *Solanum villosum* to produce the extracts and other phytochemicals without depending on the wild source which could be not available in quantities and qualities all around the year. This is also, in order for commercial production for medicinal purposes from the *Solanum villosum* plant.

5.0 Conclusion

In conclusion, the in vitro *Solanum villosum* plant as an important wild genetic resource was conserved successfully via v-cryoplate technique for the first time. This is useful to exploit its medicinal properties as permanent in vitro sources for further isolation and investigation of its medicinal and antimicrobial activity.

Declarations

6.0 Authors Contribution Statement

Shibli R. Had been contributed in supervising all over the work and writing the manuscript, **Al- Qudah T** had been contributed in doing experimental work, establishment the plant materials and writing , **Zatimeh A.** had been contributed in supervising all over the work and writing manuscript, **Tahtamouni R.** had been contributed in supervising all over the work and writing manuscript, **Abu Mallouh S** had been contributed in doing antimicrobial work.

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Availability of data and materials: The data sets supporting the results of this article will be freely available upon reasonable request.

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Figures

Figure 1

Callus culture of *Solanum vilosum* on MS media with 1.2 mg/L of Kin and 2.0 mg/L 2,4-D. Scale bar = 0.50 cm