

## Effect of Heavy Metal on the *In vitro* Growth of *Paronchia argentea* and its Antimicrobial Activity

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### ABSTRACT

*Paronchia argentea* is traditionally being used for medicinal purposes in Jordan. The current investigation was designed to check the *in vitro* efficacy of *in vitro* and *ex vitro* *P. argentea* against selected bacterial and fungal strains. The antimicrobial properties of *in vitro* plantlets and field (*ex vitro*) plant extracts of *P. argentea* were investigated against both bacteria and fungi, after and before heavy metals stress used. In this study, four bacterial species were used: *Listeria monocytogen* and *Staphylococcus aureus* (Gram positive bacteria) *Salmonella typhimurum* and *Coronobacter sakazakii* (Gram negative bacteria) and *Calvularia lunata* as a mold. The obtained results revealed that the *in vitro* grown plantlets with the supplemented of lead (Pb), copper (Cu) or Cobalt (Co) with methanol and aqueous extract showed significant inhibitory activities within zones of inhibition ranging between 6.7-30.0 mm. All extracts of *P. argentea* had activity against the fungi and bacteria tested. The maximum inhibition zone was found in *Staphylococcus aureus* (30 mm inhibition zone) in medium supplemented with 0.3 mg/L Cu followed by *Calvularia lunata* (30.0 mm inhibition zone). The methanolic and aqueous *P. argentea* extract indicate that the solvent plays an important role in the solubility of the antimicrobial substance and also affects the activity of the microbe. Both field (*ex vitro*) and tissue culture plant extract showed similar antimicrobial activity. The present study could be used as an approach for the development of new, alternative and cheap antimicrobial drugs, particularly against the infections caused by the tested microbes through the tissue culture technology.

**Keywords:** cobalt, copper, heavy metal, lead, medicinal plant, *Paronchia argentea*.

### INTRODUCTION

Caryophyllaceae is considered one of the largest family known as (pink family), which have been used in folk medicine (Atta et al., 2013). It is characterized as grassy with annual as well as has perennial growth (Böttger and Melzig, 2011). *Paronchia argentea* L. is commonly named as Rejelel-Hamama, Silver nail root or Silvery Whitlow Wort (Braca et al., 2008;

Abuhamdah, 2013). In Jordan, people are still using the medical plants for handling their illness (Pronob and Islam, 2012). *P. argentea* is used for the treatment of several disorders such as kidney stones, urinary tract infections, diseases of gastrointestinal disorders in addition to cold and fever (Noubani et al., 2006; Craker et al., 2007; Abou-Elkhir et al., 2010). The aerial parts of *P. argentea* are utilized for the treatment of abdominal pain, respiratory infections and as an anti-stress

agent (Zama et al., 2007), besides being used for stomach ulcers, bladder and prostate ailments, abdominal ailments and gastric analgesic (Ferreira et al., 2007). Abuhamdah et al. (2013) reported that crude methanolic extract of *P. argentea* had no antibacterial activity against some type of bacteria. Ferreira et al. (2007) showed that *P. argentea* can be used against flatulence, analgesic, stomach ulcers and anorexia. Moreover, it can be used for the treatment of renal diseases (Adjaj et al., 2017), hypoglycaemic activity and antimicrobial activity (Carmona et al., 2005). In addition, the *P. argentea* plant extracts have been used as a natural antioxidants and therapeutic agents for hyperuricemia (Muti and Olimat, 2018).

Heavy metals are important to plants, as they are the major unit of various enzymes. Metals such as Cr, Cu, Mn, and Fe can also directly generate oxidative injury, which leads to the formation of oxygen free radicals species in plants, resulting in cell homeostasis disruption (Kumar and Sharma, 2018). Due to better living conditions, the demand for traditional medicines and functional food sourced from medicinal plants has been rising. They are mostly grown in home gardens, and some of them are planted in more organised areas, such as sole cropping or intercropping systems and rarely as plantation crops (Hung and Chi, 2014). While the main collection method is from the wild, it has adversely affected sustainable utilisation of medicinal plants by accelerating biodiversity loss and extinction rates of endangered plant species (Li, 2015). Many plants have been used because they contain substances like phenolic compounds, synthesized by the plants. However, up to date, no work has been reported on the effect of the plant tissue extract of *in vitro* grown of *P. argentea*. Thus, *P. argentea* is considered as one of the most utilized species, but this has increased the demand on *P. argentea* which created a vital need to enhance the mass production by tissue culture. The tissue culture methods would offer a sustainable production system for this valuable plant that can be utilized for future medicinal research and industry (Shatnawi 2011a,b; Shatnawi et al., 2019; Al-Ajlouni et al., 2012; 2015). The *in vitro* culture of *P. argentea* can solve propagation problems, guaranteeing mass production of plant material without threatening natural resources; also, it improves and conserves this plant. Therefore, this study was conducted to study the growth response of *P. argentea* to heavy metal stress (Lead (Pb), Cobalt

(Co), or Copper (Cu) and to evaluate its antibacterial and fungal activity of tissue culture (*in vitro*) and field (*ex vitro*) extract of plants.

## MATERIAL AND METHODS

### Plant material

*In vitro* microshoots of *P. argentea* were obtained from Hamdi Mango Center (HMC) from Plant Biotechnology Laboratories/Faculty of Agriculture, Jordan University, Amman, Jordan. Microshoots were multiplied according to the method developed by Shatnawi et al. (2019). Microshoots were incubated at  $24 \pm 2$  °C with a 16 h photoperiod and photosynthetic photon flux density (PPFD) of  $50 \mu\text{mol m}^{-2}\text{s}^{-1}$  supplied by cool white fluorescent lamps.

### Effect of heavy metals (Pb, Co or Cu)

Plant material were subcultured onto a full strength MS callus maintenance media supplemented with lead (Pb), copper (Cu) or cobalt (Co) at different concentrations (0.0, 0.1, 0.2, or 0.3 mg/L). For each replicate, 75 mL of the medium aliquot was dispensed into a 250 mL flask. The media was solidified by using 8.0 g/L agar. In each replicate 3 microshoots were used and each treatment consisted of seven replicates. The culture conditions were maintained as described in the above section. The data on the number of new shoot height were collected after the 5th week.

### Antimicrobial activity

#### Selection of plant materials

*P. argentea ex vitro* and *in vitro* were dried under shade at room temperature ( $24 \pm 2$  °C). The *ex vitro* and *in vitro* shoot were then ground to fine powder and laid up in air tight bottles. The *ex vitro* and *in vitro* powdered (plant materials) were soaked in methanol (30%) for five days at 37 °C with continuous shaking (Labtech, Korea). The *ex vitro* and *in vitro* crude metabolic extract was filtered using 125 mm filter paper and then the extract was filtered using syringe filter with solvent (45  $\mu\text{m}$ ) (extra Gene, Taiwan); afterwards, it was stored at 2–8 °C for further use. For the water extract; a certain amount of powdered plant was soaked in 30% water for 4 h at 70–80 °C. The crude water extract was filtered using 125  $\mu\text{m}$

filter paper and then the solvent was filtered using syringe filter (45 µm) (extraGene, Taiwan); then stored at 2–8 °C for further use.

#### Culture conditions and microorganism strains

The microorganisms were obtained from Faculty of Agriculture and Technology Al-Balqa' Applied University, Department of Biotechnology (Table 1). The microorganism isolates were preserved in Brain Heart Infusion at –20 °C (BHI, Difco, MD, USA) and Potato Dextrose (PDA, Oxoid) for bacteria and fungi, respectively. Before any experimentation, the microorganism culture was executed to give isolate life to each culture, and then the microorganism was then transferred to BHI broth and saline solution (0.85%) added with (0.2%) Tween 80 for bacteria and fungi, respectively. The microorganisms then were kept in the growth chambers and grown at 37 °C and 27 °C for 24 and 48 h, until they reached the optimal growth (stationary phase), for both bacteria and fungi, respectively (Awaisheh and Ibrahim, 2009; Al-Turk et al. 2020).

#### Antimicrobial activity assay

An inoculum ( $10^5$  CFU/ml was prepared using McFarland techniques) was placed into nutrient agar plates (NA; Oxoid). The mold inoculums were cultured on Potato Dextrose Agar (PDA; Oxoid), using pour plate technique. For antimicrobial activity screening, the agar well diffusion assay (AWDA) was employed. About 5 mm well was inoculated using pasture pipettes (Borosilicate glass, Fisher Scientific Company; 5 mm) on each plate; then, 25 µl of each extract water or methanol were supplemented. The un-inoculated plates were used as negative controls in order to exclude any effects of these tools opposite the tested microorganisms. After 20 min at  $24 \pm 2$  °C for the plant extract to spread and transit the surface, then for one day the bacteria plates were incubated at 37 °C. For mold, it was incubated at 27 °C for 48 h. Each experiment was repeated three times for

each extract; using caliper of the inhibition zone was measured (mm) (Awaisheh et al., 2015).

#### Statistical analysis

Treatments in each experiment were arranged in a completely randomized design (CRD). In shoot multiplication experiments, each treatment was repeated three times. Analysis of variance (ANOVA) was used and a mean separated was tested at 0.05 probability level according to the Tukey's HSD. The data were statistically analyzed using SPSS analysis system.

## RESULTS

#### Effect of heavy metal

Maximum numbers of new microshoots (1.95 cm) were produced on the MS medium supplemented with 0.3 mg/L Cu. The microshoot length decreased as a result of increasing Cu concentration in the media (Table 2). In the presence of 0.3 mg/L Cu, dry weight increased along with the Cu concentration (0.1 to 0.2 mg/L Cu) (Table 2). Using the MS medium supplemented with Pb negatively affected the number of new microshoots by adding 0.1 mg/Pb to the medium (Table 3). Maximal value for fresh weight was (0.110 g) weight on media supplemented with 0.1 to 0.3 mg/L Pb. Furthermore, increasing the Pb concentration in the medium (0.3 mg/L) did not increase dry weight significantly (Table 3). Thus, higher concentrations of Pb slightly inhibited shoot proliferation as well as growth of shoots (Table 3). Table (4) shows the effect of different Co concentrations on the formation of new microshoots. The MS medium supplemented with 0.3 mg/L Co produced 1.7 microshoots per explant. The microshoot length was increased significantly as a result of the increase of the Co concentration. With increasing Co concentration in the media, there were no significant differences in fresh and dry weights (Table 4).

**Table 1.** Microorganisms that were used in this study

| Name of microorganism         | Origin                     | Type                   |
|-------------------------------|----------------------------|------------------------|
| <i>Listeria monocytogenes</i> | ATCC 9644                  | Gram positive bacteria |
| <i>Salmonella typhimurum</i>  | (ST00200) Clinical isolate | Gram negative bacteria |
| <i>Staphylococcus aureus</i>  | ATCC 25923                 | Gram positive bacteria |
| <i>Coronobacter sakazakii</i> | Clinical isolate           | Gram negative bacteria |
| <i>Calvularia lunata</i>      | Clinical isolate           | Mold                   |

**Table 2.** Effect of different Cu concentrations on *in vitro* *Paronychia argentea* after five weeks on MS media added with 0.2 mg/L BAP

| Cu concentration (mg/L) | Number of microshoots | Microshoot length (cm) | Fresh weight (g) | Dry weight (g) |
|-------------------------|-----------------------|------------------------|------------------|----------------|
| 0.0                     | 1.70a                 | 2.8a                   | 0.090a           | 0.020a         |
| 0.1                     | 1.62a                 | 3.3b                   | 0.090a           | 0.018a         |
| 0.2                     | 1.61a                 | 2.6a                   | 0.095a           | 0.018a         |
| 0.3                     | 1.95b                 | 2.7a                   | 0.095a           | 0.018a         |

Values represent means; each treatment consisted of three treatments, with seven microshoots each treatment, each experiment repeated twice. Means within a column test based on Tukey's HSD test at  $P \leq 0.05$ . Data were collected after five weeks growth periods.

**Table 3.** Effect of different Pb concentrations on *in vitro* *Paronychia argentea* after five weeks on MS media added with 0.2 mg/L BAP

| Pb concentration (mg/L) | Number of microshoot | Microshoot length (cm) | Fresh weight (g) | Dry weight (g) |
|-------------------------|----------------------|------------------------|------------------|----------------|
| 0.0                     | 1.71b                | 2.39a                  | 0.095a           | 0.020a         |
| 0.1                     | 1.58b                | 2.89b                  | 0.110a           | 0.018a         |
| 0.2                     | 1.14a                | 2.77b                  | 0.110a           | 0.018a         |
| 0.3                     | 1.11a                | 2.80b                  | 0.110a           | 0.018a         |

Values represent means; each treatment consisted of three treatments, with seven microshoots each treatment, each experiment repeated twice. Means within a column test based on Tukey's HSD test at  $P \leq 0.05$ . Data were collected after five weeks growth periods.

**Table 4.** Effect of different Co concentrations on *in vitro* *Paronychia argentea* after five weeks on MS media added with 0.2 mg/L BAP

| Co concentration (mg/L) | Number of microshoots | Microshoot length (cm) | Fresh weight (g) | Dry weight (g) |
|-------------------------|-----------------------|------------------------|------------------|----------------|
| 0.0                     | 1.70a                 | 2.2a                   | 0.090a           | 0.020b         |
| 0.1                     | 1.62a                 | 2.3b                   | 0.090a           | 0.020a         |
| 0.2                     | 1.72a                 | 3.8c                   | 0.100a           | 0.020a         |
| 0.3                     | 1.70a                 | 3.8c a not b           | 0.100a           | 0.020a         |

Values represent means; each treatment consisted of three treatments, with seven microshoots each treatment, each experiment repeated twice. Means within a column test based on least significant difference (LSD) at 0.05 level of probability. Data were collected after five weeks growth periods.

The influence of aqueous and methanolic extracts of *P. argentea* (*ex vitro* and *in vitro*) against different bacterial and fungal strains is Drought Stress shown in Tables 5, 6, 7 and 8. In this study, four bacterial species were used: *Listeria monocytogen* and *Staphylococcus aureus* (Gram positive (G +ve) bacteria) *Salmonella typhimurum* and *Coronobacter sakazakii* (Gram negative (G -ve) bacteria) and *Calvularia lunata* as a mold. Aqueous and methanolic extracts showed variable antimicrobials *Salmonella typhimurum* and *Coronobacter sakazakii* (Table 5, 6, 7 and 8). Maximum *ex vitro* antimicrobial activity of plants were against *Salmonella typhimurum* and *Coronobacter sakazakii* ( $30.0 \pm 0.2$ ) using aqueous or methanolic extract (Table 5). Drought stress *in vitro* plant extracts did not show any inhibitory activity to *Staphylococcus aureus*. Moreover, *in vitro*

methanolic extract did not show any inhibitory activity to *Salmonella typhimurum*, *Listeria monocytogen* and *Staphylococcus aureus*. Out of the five microorganisms used, *Salmonella typhimurum* and *Listeria monocytogen* were the most sensitive to the *ex vitro* methanolic extract (Table 5).

The aqueous and methanolic plant extracts from the *ex vitro* and *in vitro* plantlet proved to be very effective against *Calvularia lunata* with maximum inhibition zone of 30.0 mm (Tables 5). Therefore, the plant extract showed significant antifungal activity against the strain tested in this study. Among the plant extracts, the *ex vitro* plant extract showed greater inhibition towards all the fungi strains. Both *ex vitro* and *in vitro* plantlets showed similar antifungal properties (Table 5).

Table 6 shows the antibacterial and antifungal activity of *P. argentea* methanolic and

**Table 5.** Antimicrobial activities of *Paronychia argentea* extract against five microorganisms of *in vitro* and *ex vitro* plants using methanol and aqueous extract

| Test microorganism            | <i>Ex vitro</i> plant |                  |                         |            |
|-------------------------------|-----------------------|------------------|-------------------------|------------|
|                               | Positive control      | Negative control | Methanol                | Aqueous    |
| <i>Salmonella typhimurum</i>  | 15.0 ± 0.0 (AMP)      | 0.00±0.00        | 30.0±0.10               | 30.00±0.10 |
| <i>Listeria monocytogen</i>   | 20.0 ± 0.1(STR)       | 0.00±0.00        | 10.0±2.516              | 15.0±0.10  |
| <i>Staphylococcus aureus</i>  | 30.0 ± 0.0 (P)        | 0.00±0.00        | 20.0±0.20               | 14.70±0.10 |
| <i>Coronobacter sakazakii</i> | 30.0 ± 0.0 (AM)       | 0.00±0.00        | 30.0±0.00               | 30.0±0.103 |
| <i>Calvularia lunata</i>      | 30.0 ± 0.1(AB)        | 0.00±0.00        | 30.0±0.00               | 25.0±0.10  |
| <i>In vitro</i> plantlets     |                       |                  |                         |            |
| <i>Salmonella typhimurum</i>  | 15.0 ± 0.0 (AMP)      | 0.00±0.00        | 00.0 ± 0.0              | 00.0 ± 0.0 |
| <i>Listeria monocytogen</i>   | 20.0 ± 0.1(STR)       | 0.00±0.00        | 00.0 <sup>1</sup> ± 0.0 | 00.0 ± 0.0 |
| <i>Staphylococcus aureus</i>  | 30.0 ± 0.0 (P)        | 0.00±0.00        | 00.0 ± 0.0              | 00.0 ± 0.0 |
| <i>Coronobacter sakazakii</i> | 30.0 ± 0.0 (AM)       | 0.00±0.00        | 20.0 ± 0.2              | 10.0 ± 0.4 |
| <i>Calvularia lunata</i>      | 30.0 ± 0.1(AB)        | 0.00±0.00        | 25.0 ± 0.1              | 20.0 ± 0.1 |

The positive controls for bacterial strains were streptomycin (STR), ampicillin (AM), Ampicill (AMP) penicillin (P). For mold strain Amphotericin B (AB) were used as control positive.

**Table 6.** Antimicrobial activities of *Paronychia argentea* extract against five microorganisms of *in vitro* plants using methanol and aqueous extracts grown on MS medium added with different Copper (Cu) concentrations

| Test microorganism            | Copper (Cu) |            |            |
|-------------------------------|-------------|------------|------------|
|                               | Methanol    |            |            |
|                               | 0.1         | 0.2        | 0.3        |
| <i>Salmonella typhimurum</i>  | 0.00±0.00   | 0.00±0.00  | 0.00±0.00  |
| <i>Listeria monocytogen</i>   | 0.00±0.00   | 0.00±0.00  | 0.00±0.00  |
| <i>Staphylococcus aureus</i>  | 15.0±0.02   | 25.0±0.002 | 30.0±0.002 |
| <i>Coronobacter sakazakii</i> | 0.00±0.00   | 0.00±0.00  | 0.00±0.00  |
| <i>Calvularia lunata</i>      | 10.0±0.006  | 25.0±0.00  | 30.0±0.001 |
| Aqueous                       |             |            |            |
| <i>Salmonella typhimurum</i>  | 0.00±0.00   | 0.00±0.00  | 0.00±0.00  |
| <i>Listeria monocytogen</i>   | 0.00±0.00   | 0.00±0.00  | 0.00±0.00  |
| <i>Staphylococcus aureus</i>  | 0.00±0.00   | 7.70±0.003 | 10.0±0.003 |
| <i>Coronobacter sakazakii</i> | 0.00±0.00   | 0.00±0.00  | 0.00±0.00  |
| <i>Calvularia lunata</i>      | 0.00±0.00   | 7.70±0.006 | 14.7±2.561 |

Results are Mean ± standard error of mean (SEM) of three determinations of two independent experiments.

aqueous extract in the medium supplemented with different concentrations of copper (Cu). Out of the four bacterial isolates used in this experiment, isolated *Staphylococcus aureus* exhibited the antimicrobial activity and it is antimicrobial activity increased along with Cu concentration in the medium when both extract were used. By using Cu with methanolic extract, different antifungal activities were observed, ranging from 10–30 mm zones of inhibition. Moreover, similar results were obtained on *Calvularia lunata* with the aqueous extract was used, showing inhibition zones ranging 7.6–14.6 mm (Table 6).

Table 7 showed that antimicrobial activity of *P. argentea* against five microorganisms of *in vitro* plantlets using methanol and aqueous extract grown on MS medium supplemented with different lead (Pb) concentrations. The medium containing 0.1 mg/L Pb did not show any growth inhibition (0.0 mm) for *Listeria monocytogen* and *Staphylococcus aureus*, *Salmonella typhimurum* and *Coronobacter sakazakii* except for *Calvularia lunata* (10 mm inhibition zone). In turn, the aqueous extract at concentration of 0.3 mg/L showed increased inhibition zone for *Salmonella typhimurum* (10.0 mm inhibition zone), *Staphylococcus aureus* (25.0 mm inhibition zone) and

**Table 7.** Antimicrobial activities of *Paronychia argentea* extract against five microorganisms of *in vitro* plants using methanol and aqueous extracts grown on MS medium supplemented with different Lead (Pb) concentrations

| Test microorganism            | Lead (Pb)   |            |             |
|-------------------------------|-------------|------------|-------------|
|                               | Methanol    |            |             |
|                               | 0.1         | 0.2        | 0.3         |
| <i>Salmonella typhimurum</i>  | 0.00±0.00   | 0.00±0.00  | 14.7±2.52   |
| <i>Listeria monocytogen</i>   | 0.00±0.00   | 0.00±0.00  | 0.00±0.00   |
| <i>Staphylococcus aureus</i>  | 0.00±0.00   | 0.00±0.00  | 0.00±0.00   |
| <i>Coronobacter sakazakii</i> | 0.00±0.00   | 0.00±0.00  | 7.7±0.34    |
| <i>Calvularia lunata</i>      | 10.0±0.34   | 14.6±2.51  | 25.0 ±0.001 |
|                               | Aqueous     |            |             |
| <i>Salmonella typhimurum</i>  | 4.70± 0.012 | 7.70±0.34  | 10.0±0.006  |
| <i>Listeria monocytogen</i>   | 0.00±0.00   | 0.00±0.00  | 0.00±0.00   |
| <i>Staphylococcus aureus</i>  | 10.0± 0.002 | 14.7±0.012 | 25.0±0.006  |
| <i>Coronobacter sakazakii</i> | 0.00±0.00   | 0.00±0.00  | 0.00±0.00   |
| <i>Calvularia lunata</i>      | 14.7±0.012  | 20.0±0.003 | 25.0±0.001  |

Results are Mean ± standard error of mean (SEM) of three determinations of two independent experiments.

**Table 8.** Antimicrobial activities of *Paronychia argentea* extract against five microorganisms of *in vitro* plants using methanol and aqueous grown on MS medium added with different Cobalt (Co) concentrations

| Test microorganism            | Cobalt (Co) |             |             |
|-------------------------------|-------------|-------------|-------------|
|                               | Methanol    |             |             |
|                               | 0.1         | 0.2         | 0.3         |
| <i>Salmonella typhimurum</i>  | 0.00±0.00   | 15.0 ±2.516 | 17.0±0.34   |
| <i>Listeria monocytogen</i>   | 0.00±0.00   | 0.00±0.00   | 0.00±0.00   |
| <i>Staphylococcus aureus</i>  | 14.7±2.516  | 20.0±0.006  | 25.0±0.001  |
| <i>Coronobacter sakazakii</i> | 0.00±0.00   | 0.00±0.00   | 0.00±0.00   |
| <i>Calvularia lunata</i>      | 14.7±2.516  | 20.0±0.006  | 25.0±0.001  |
|                               | Aqueous     |             |             |
| <i>Salmonella typhimurum</i>  | 0.00±0.00   | 0.00±0.00   | 0.00±0.00   |
| <i>Listeria monocytogen</i>   | 0.00±0.00   | 0.00±0.00   | 0.00±0.00   |
| <i>Staphylococcus aureus</i>  | 10.0± 0.002 | 20.0±0.006  | 25.0±2.561  |
| <i>Coronobacter sakazakii</i> | 0.00±0.00   | 0.00±0.00   | 0.00±0.00   |
| <i>Calvularia lunata</i>      | 10.0± 0.002 | 20.0±0.003  | 25.0 ±0.003 |

Results are Mean ± standard error of mean (SEM) of three determinations of two independent experiments.

*Calvularia lunata* (25 mm inhibition zone) with methanolic extract (Table 7). The aqueous extract exhibited higher activities with *Salmonella typhimurum* (10.0 mm inhibition zone), *Staphylococcus aureus* (25.0 mm inhibition zone) and *Calvularia lunata* (25 mm inhibition zone) (Table 6). In the medium supplemented with water extracts and methanolic extract, no reasonable activity against *Listeria monocytogen* was demonstrated (Table 6).

Table (8) shows the influence of different concentrations of Co on the antibacterial and antifungal activities of the *in vitro* *P. argentea* methanolic and aqueous extracts. Both

methanolic and aqueous extracts showed no inhibition activity against *Listeria monocytogenes* and *Coronobacter sakazakii*. *P. argentea* was effective against *Salmonella typhimurum*, the diameter of maximum inhibition zone ranged between 15–17 mm when Co was used at 0.2–0.3 mg/L, respectively (Table 8). The extract of *P. argentea* also showed the antifungal activity against *Calvularia lunata*. Inhibition zone diameters were between 10–25 mm. Both extract showed antifungal property in the medium supplemented with Pb, Cu or Co. The extracts showed concentration-dependent antibacterial and antifungal properties (Table 8).

## DISCUSSION

### *In vitro* growth

Tissue culture is a very useful technique to study propagation in aseptic and artificial environment with various industries to produce plantlets for sustainable farming (Nawrot-Chorabik, 2014; Wiszniewska et al., 2015; Yong et al., 2017). By using plant tissue culture, fast growing, genetically true to type plant can be produced that can be tolerant or pollutant in a short period of time and such plants can be used for the phytoremediation purposes (Nawrot-Chorabik, 2016). Therefore, one of the objectives of the current study was to evaluate the effect of different concentrations of Pb, Cu or Co on the *in vitro* growth of *P. argentea* (Table 2, 3 and 4).

The load of heavy metal toxins in the world has been increased due to heavy industry (Wao et al., 2014; Wiszniewska et al., 2015). However, the plant material that can be used for phytoremediation in any stress condition should have ability to produce a large amount of fresh weight (Wao et al., 2014). Heavy metal accumulation had negative effects on plant productivity due to heavy metal phytotoxicity (Wao et al., 2014). With high industrial development, such heavy metals as Cu, Pb or Co are major environmental pollutants (Alkorta et al., 2004). Moreover, plantlet dry weight strongly depends on the nutrient uptake under stress conditions (Wiszniewska et al., 2015). Kabata-Pendias and Mukherjee (2007) reported that Pb had a negative impact on the growth and the uptake of P, K, Ca, Cu, Fe, Mn and Zn. The current study showed that Pb had a negative impact on the *in vitro* *P. argentea* plantlets. With increasing Co concentrations in the media, there were no significant differences in fresh and dry weights (Table 4). The alternation in fresh weight was not significant at any point of growth due to the presence of Co. Copper is essential for plant growth and development, since it interacts with many enzymes and proteins (Wiszniewska et al. 2015). Yong et al. (2017) mentioned that Co is considered as a transition element which plays an important role in plants as an enzyme cofactor absorbed from soil or media by tissues or plants. The current study confirmed that *in vitro* method could be used to study the impact of heavy metals on microshoots of *P. argentea* in culture media containing known quantities of specific heavy metal (Wiszniewska et al., 2015).

### Antimicrobial activity

Medicinal plants are supposed to be rich in the components which can be used in development and synthesis of drugs. These compounds found in plants are in four biochemical groups, such as glycosides, polyphenols, alkaloids and terpenes (Abou Elkhair et al., 2010; Obaidat et al., 2012; Grzeszczuk et al., 2018). Therefore, scientists are looking to develop new antimicrobial compounds from different natural sources such as animal, plants and micro-organisms. Several plants extracts are used to treat numerous infections, as they have the antimicrobial energetic potential. This is an important issue in searching for a new source of antimicrobial material as they produce a variety of bioactive compounds of known therapeutic properties (Faizi et al., 2003).

The results of the current study showed that *P. argentea* had high potential that can be used as an antimicrobial medicinal plant (Tables 5, 6, 7 and 8). The *P. argentea* extract containing active compound could pass through the cell wall which may slow the growth of the microorganism (Obaidat et al., 2012). Therefore, the current study verifies the use of methanolic and aqueous extract. Moreover, different microorganisms show variable sensitivity (Abou Elkhair et al., 2010; Obaidat et al., 2012). *P. argentea* had considerable antibacterial and antifungal properties. The antibacterial and antifungal properties of *P. argentea* in the extract are probably the motive to the presence of “oleanane saponins (1 and 2) and one new flavonol glycoside (3) together with six known flavonoids” (Braca et al., 2007). The previous chemical analysis showed that *P. argentea* contains flavonoids, steroids and saponins (Abou Elkhair et al., 2010).

*P. argentea* has an economic and ecological value such as grazing, food recipes, land escaping and sand stabilization (Bidak et al., 2014). However, negative results (zero inhibition zone) in Table (5 to 8) did not mean the absence of bioactive compound in the plant. The activity can thus only be proven by using large doses; this is similar to our finding in this study (Obaidat et al., 2012, Alrayes et al., 2016). Alternatively, if the active chemical compound presents in high enough quantities there could be other disputing effects (Jnoviska et al., 2003; Odat 2020). In this study, no antibacterial activity was reported for some species; this means that the extracts may be

effective against different other bacterial species (Yadav et al., 2008). Thakare et al. (2016). reported that many natural products such as enzymes, pigments and bioactive components, are soluble in water, which explains the effect of the aqueous extract. Most of the solvent enhanced the antimicrobial activity of the extracts.

The extract of *ex vitro* plant of *P. argentea* showed maximum activity against *Salmonella typhimurum* or *Coronobacter sakazaki* (30 mm zone of inhibition), followed by *Staphylococcus aureus* and (20 mm zone of inhibition). The *ex vitro* and *in vitro* extracts exhibited antibacterial properties against the bacteria under study. Both aqueous and methanolic extracts were found to be effective for all fungi and bacteria tested in this study. Both extracts were effective against *Calvularia lunata* (fungi) and the activity was similar to the antifungal agent tested. Both aqueous and methanolic extract species showed inhibition against *P. argentea*. This indicated that the methanolic solvent plays an important role in the solubility of the antimicrobial substance and which may be affects the microbial activity. However, many researchers try developing less toxic plant extracts effective in controlling the microorganism growth (Sena and Barata, 2012). In the current studies, the tested bacteria were inhibited by methanolic or aqueous extracts.

Our results revealed that the *in vitro* plant supplemented with Pb, Cu, Co or NaCl with methanol and aqueous extract showed significant inhibitory activities within zones of inhibition ranging from 6.7–30.0 mm (Tables 5, 6, 7 and 8). All extracts of *P. argentea* showed the activity against fungi. This activity was closed to the antibiotic tested for each bacterial species (Table 13). The maximum inhibition zone was found in *Staphylococcus aureus* (30 mm inhibition zone) in the medium supplemented with 0.3 mg/L Cu, followed by *Calvularia lunata* (30.0 mm inhibition zone). Methanolic and aqueous *P. argentea* extract indicate that the solvent plays an important role in the solubility of the antimicrobial substance and also affects the activity of the microbe.

The *P. argentea* plants have been reported to contain many phytoconstituents such as alkaloids, flavonoids, inulin tannins, steroids volatile oil, and triterpenoids (Barca et al., 2013). The methanolic extracts have more antimicrobial energetic compared with aqueous extracts. This is because the non-polar residues in plant extracts have high

prohibitive effect against different microorganisms tested in this study (Sen and Barata, 2012). A previous study by Adzu (2003), showed that the ethanol extract of *P. argentea* affected permeability the intestine through the inhibition of prostaglandins release. Thus, the results showed that the extracts from *P. argentea* possessed the antimicrobial activity. *In vitro* plantlets may contain secondary metabolites resulting in the antimicrobial property. Therefore, this important plant can be subjected to pharmacological evaluation to obtain safe and cheap drugs.

*P. argentea* (*ex vitro* or *in vitro*) contains broad spectrum of active compounds known by their active substances constituents such as alkaloids, flavonoids, inulin tannins, steroids volatile oil, and triterpenoids. The secondary metabolites from plant extracts have been found to possess high antimicrobial and antioxidant properties in various studies (Lonare et al., 2012; Mitrovic et al., 2014). The alkaloids are also effective in their antimicrobial (Manandhar et al., 2019) and antioxidant (Mitrovic et al., 2014) activities. In conclusion, the *P. argentea* plants extract showed the possibility for use as antimicrobial agents. The methanolic extracts exhibited high antimicrobial activity as compared with the aqueous plant extract. The *P. argentea* plants have to be subjected to phytochemical and pharmacological studies followed by the identification of the active compounds.

## CONCLUSIONS

The *in vitro* grown plantlets supplemented with lead (Pb), copper (Cu), or cobalt (Co) with methanol and aqueous extract displayed substantial inhibitory activities within zones of inhibition ranging from 6.7–30.0 mm, according to our findings. All extracts of *P. argentea* exhibited activity against fungi and bacteria tested. *Staphylococcus aureus* had the largest inhibition zone (30 mm) in the medium supplemented with 0.3 mg/L Cu, followed by *Calvularia lunata* (30.0 mm inhibition zone). The solvent plays an important role in the solubility of the antimicrobial substance and also affects the activity of the microbe in methanolic and aqueous *P. argentea* extracts. The current study could be used to develop new, alternative, and low-cost antimicrobial drugs, specifically against the infections caused by the tested microbes, using the tissue culture technology.

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