INTRODUCTION

Medicinal as well as agriculturally important plants of Jordan are under threat due to several factors of habitat perturbation such as drought and salinity (AL-Issa et al. 2020; Odat, 2020). Additionally, other factors include ruinous over-harvesting, climate change, competition and invasion. Medicinal plants have been used as source of medication for many years. They have been used for centuries due to their antihypertensive, anti-asthmatic, anti-diabetic, as well as antimicrobial activities and today their scientific justification was provided by recognition and isolation of bioactive phytochemicals (Candan et al., 2010; Faiku et al., 2018). In Jordan, medicinal plants constitute an important component of Jordanian flora, because of its geographic location and favorable climatic conditions. Moreover, different plant parts have been used naturally for treating a variety of microbes. Plants that were historically used in medicine as a result of indigenous people’s expertise devise. This knowledge contributes the discovery of novel drugs, which provide new approach to the research activity (Oriabi, 2016). Increased demand on plants and growing population involved in illegal collection of medicinal plants cause exhaustion of medicinal plant and endanger their natural habitat.

The demand of medicinal plants has increased along with the use of plants in pharmaceutical companies and for exploring the possibilities of finding active ingredient. Therefore, the prices of medicinal plant and their products are increasing.
due to high demand. Hence, the availability of medicinal plant in nature decreased and the deprivation of plant species is increasing. Jordan is one of the highest regions in the world in which medicinal plants grown (Obeidat et al., 2012; Oriabi, 2016). Moreover, many medicinal plant species are becoming endangered at alarming rate due to rapid development in agricultural and urban, collection and deforestation (Islam et al., 2005).

Using conventional propagation methods, some medicinal plants do not germinate, or do not produce seed, or have long periods of growth and multiplication (Shatnawi et al., 2013; 2019). The in vitro-propagated medicinal plants could provide a source of uniform plants, and plants with high multiplication rate (Shatnawi et al., 2011; Alrayes et al., 2016). The in vitro plant technique could be proven alternative method for the propagation of plant material that is needed for the conservation of medicinal plants (Vitalini, et al., 2011; Haque et al., 2017). This technology is mainly based on propagation of plant culture in vitro from cutting of axillary buds, somatic embryos, callus or suspension culture. Thus, the tissue was used as a research tool and focused on the attempts to culture and study the development of small, plant tissues or isolated cells (Stephenson and Fahey, 2004; Bollana et al., 2020). Moreover, producing plant secondary metabolites under controlled environment is considered reliable under controlled environment condition. In addition, purification and extraction of plant secondary metabolites from tissue cultures is easy which minimizes the cost of purifying and producing such valuable compounds (Al Khateeb et al., 2013). Therefore, the aims of this study were to promote protocol for in vitro propagation as well as to evaluate the antimicrobial activities of Achillea millefolium and Moringa peregrina in vitro plantlets and ex vitro plants.

MATERIAL AND METHODS

Culture conditions and plant material

The investigations of the current study were carried out in Al-Balqa applied university. The seeds of Achillea millefolium Jerash (Latitude: 32° 16' 36.84" N; Longitude: 35° 54’ 20.16), while Moringa peregrina were obtained from South of Dead Sea, Jordan (31° 74’880" N; 35° 59’378”E) were used in this study. In the multiplication and rooting stages, glass flasks (250 mL) containing 75 mL of solid medium 7 g/L agar agar were used. Four to five explants were placed per each flask. The plant material was incubated in an artificial light chamber (photoperiod 16 h light and 8 h darkness, light intensity: 45–50 μmol m$^{-2}$s$^{-1}$ at 24±2 °C.

In Vitro establishment

Healthy plants were selected, from which the seeds were collected. Disinfection was carried out with sodium hypochlorite at 4.0% (m/v) for 20 min with the addition of two drops from Tween 20. The seeds were planted in water media with the addition of 7g/L agar. Germination of seed was evaluated on a water medium under light condition. Microshoots were then subculture into Murashige and Skoog (MS) medium (1962) with the addition of 0.05 mg/L 6-benzyl amino purine (BAP) with sucrose 30 g/L. Then, pH was adjusted to 5.8–5.9, with 8 g/L agar agar supplementary before autoclaving at 121°C for 20 min. They were then placed in the dark or artificial light chamber (15 seeds per treatment). After four weeks, the percentage of germination and contamination were evaluated.

Shoot proliferation

The microshoots with fifteen mm in length with apical meristem obtained from the germinated in vitro seeds were inoculated in MS medium, containing different BA concentration (0.0, 0.3, 0.6, 1.0, 1.2 or 1.5 mg/L). In order to avoid the carryover effect of the growth regulators, the microshoots were subcultured onto hormone free MS medium for four weeks. Into each 250 mL flask, eighty mL of the medium were dispensed. After six weeks, the data were collected on number of shoots per explants and number of microshoot. Ten replicates were used for each treatment and each flask consisted of three microshoots.

Antimicrobial activity

The antimicrobial activities of the two plants (A. millefolium and M. peregrina) extracts were tested. On solid media in Petri plates, the antimicrobial activity was tested according to the methods developed by Alrayes et al. (2016). For the bacterial assay, Muller Hinton (38 gm/L), and for
fungus, PDA (39 gm/L), the media (about 15 ml) were poured to 9 cm sterile Petri dishes to obtain 0.4 cm thickness. The media were used for developing surface colony growth. The MIC, the minimum inhibitory concentration and the MBC the minimum bactericidal concentration, were determined by the serial micro-dilution assay.

Tested bacterial species

In this study, different bacterial species were evaluated (*Klebsiella oxytoca* ATCC 18182, *Salmonella paratyphi* ATCC 13076, *Staphylococcus aureus* ATC 29974, *Klebsiella oxytoca* ATCC18182, *Proteus vulgaris* ATCC 13315, and *Proteus mirabilis* ATCC 112453). The bacterial cultures were obtained from Dr. Maher Obeidat from the laboratory of Biotechnology at Al-Balqa Applied University, Jordan. At 4 °C the bacterial cultures were preserved on a nutrient broth medium.

Plant extract

The *ex vitro* (field leaf plants) and *in vitro* (plantlet) plant materials of *Achillea millefolium* and *M. peregrina* were dried for fourteen days in the shade, then ground to a fine powder by using a blender, according to the methods developed by Alrayes et al. (2016). For water extract 30 gram of the grinded powder was weighed in 500 ml Erlenmeyer flask of to which 100 ml distilled water was added for pre-extraction and kept in water path at 70 °C overnight, then the resulting mixture was filtered using a sterile Muslin cloth and Whatman No 1 filter paper and finally with 0.45 ul filter, stored in sterile bottle. For the methanol, ethanol and acetone extract, 30 gram of the ground powder was weighed in 500 ml Erlenmeyer flask to which 100 ml of absolute, ethanol methanol and acetone, were added for the pre extraction the conical flasks were shaken intermittently for seven days at room temperature. Then, the resulting mixture was filtered using a sterile Muslin cloth and Whatman No 1 filter paper, then the solvents were removed by evaporation, the residue forms were weighed, labeled, then dissolved in dimethyl sulphoxide (DMSO) and stored in sterile bottles and kept in refrigerator and evaluated for their ability to inhibit the bacterial and fungal growth. Extracts from all these extraction methods were tested for purity by plating them on Mueller Hinton Agar (MHA) (Oxoid, UK) and incubated for 24 hours at 37°C.

Antibacterial activity assay

For the evaluation of antibacterial activity, the cultures of bacteria were adjusted to 0.5 McFarland turbidity standard (Ataee et al., 2012). Within 15 minutes after adjusting the turbidity of the inoculums suspension this basic suspension should contain approximately 107 to 108 CFU/ml, a sterile non-toxic swab on an applicator was dipped into the adjusted suspension. The swab was rotated several times, and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. Then the dried surface of the Muller Hinton agar plate was streaked by the swab over the entire sterile agar surface, (this streaking procedure was repeated two more times).

Agar surface was bored by using sterilized gel borer to make wells (7 mm diameter). Then, 100 µl of the plant extract at (30 mg/100 µl for water extract and 40 mg/100 µl for organic extracts), and 100 µl of sterilized distilled water or dimethyl sulfoxide (DMSO) (negative control) were poured in to separate wells. The standard antibiotic disc kanamycin (30 µ/disc), was placed on the agar surface as positive control and left for one hour to diffuse. Then, the plates were incubated aerobically in upright position at 37±2 °C for 18–24 h for bacterial pathogens. Control experiments comprising inoculate without plant extract were set up. The antimicrobial activity was determined by measuring zone of inhibition around at the end of the incubation period. The results were compared with the control antibiotic, kanamycin. For each extract, three replicates were conducted against each organism.

Statistical analysis

All experiments were performed in a completely randomized design. Analysis of Variance (ANOVA) was employed for parametric tests. Tukey HSD was used for mean separation at $P=0.05$. The data were analyzed using SPSS (version 20.0).

RESULTS

Effect of BA on microshoots proliferation of *A. millefolium* and *M. peregrina*

Table 1 shows the influence of BA on *in vitro* microshoot multiplication of *A. millefolium*
and *M. peregrina*. BA once used for multiplication permitted the growth of new shoots for each explant. However, for shoot proliferation, there were optimistic responses to BA. Maximum rate multiplication for *M. peregrina* was gained by using 0.6 mg/L BA (5.8 microshoots), while for *A. millefolium* (6.1 microshoot) it was at 1.0 mg/L BA (Table 1).

**Antimicrobial activity of *A. millefolium* and *M. peregrina***

In this study, the ethanolic extract of *A. millefolium* and *M. peregrina* was investigated to assess whether *ex vitro* (field plants) *in vitro* (tissue culture plant) will retain the same activity or not (Table 3). The ethanol and aqueous extracts of two medicinal plants were tested against the investigated bacterial strains (*Klebsiella oxytoca*, *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella paratyphi*). In this study, assay of both (aqueous and ethanol) extracts showed wide range of zone inhibition. The antimicrobial activity of *A. millefolium* and *M. peregrina* extract may be due to the presence of phenolic (secondary metabolites) constituents. *M. peregrina* contains organic extracts in the range of 11 and 22.83 mm. This is due to the presence of little amount of antimicrobial compounds in the plant cell (secondary metabolites).

There are rare finding about the antimicrobial activity applied by *A. millefolium* and *M. perigrina* ethanolic extract. The assay of the extracts showed that the ethanol and aqueous extracts revealed a wide-ranging degree, opposite to bacteria (Table 2). In general, the *ex vitro* or *in vitro* ethanol extracts of *M. peregrina* presented more effect than aqueous extracts and produced zones of inhibition ranging from 22.3 for *Staphylococcus aureus* to *Klebsiella oxytoca* for 12.3 mm. The ethanolic *in vitro* extracts of *M. peregrina* showed the highest degree of inhibition against *Staphylococcus aureus* (23.3 mm), *Salmonella paratyphi* (22.5), *Proteus mirabilis* (18.2 mm), *Proteus vulgaris* (13.3 mm) and *Klebsiella oxytoca* (13.3 mm) (Table 3). *S. aureus* (19.5 mm inhibition zone) was revealed to be the most sensitive, and the zone of inhibition showed by the *ex vitro* ethanolic extract of *M. peregrina* was 23.3 mm. Additionally, the ethanolic extracts were reported to be higher than the aqueous extracts. The highest inhibition (22.5 mm) was detected in the *ex vitro* ethanolic extract with *Salmonella paratyphi* (Table 3).

Assessment of the antimicrobial activity of *A. millefolium* plant extracts was tested originally by using aqueous and ethanolic extracts against different microorganisms. It was observed that *Salmonella paratyphi* was the most effective among the five bacteria tested to both *ex vitro* and *in vitro* plant extract (Table 4 and 5). Using *ex vitro* ethanolic extract of *A. millefolium* showed higher grades of inhibition against *Staphylococcus aureus* (19.8 mm), *Salmonella paratyphi* (19.7 mm), *Proteus mirabilis* (13.9 mm),

| Growth regulator (mg/L) | Shoot number | Shoot length (mm) |
|------------------------|--------------|-------------------|
|                         | *Achillea millefolium* |               |
| 0.0                    | 1.90 c        | 45.5 b            |
| 0.3                    | 3.75 c        | 50.9 a            |
| 0.6                    | 5.0 b         | 52.8 a            |
| 1.0                    | 6.1 a         | 46.9 b            |
| 1.2                    | 2.99 c        | 44.5 b            |
| 1.5                    | 2.35 d        | 33.8 d            |
|                         | *Moringa peregrina* |               |
| 0.0                    | 2.3 d         | 29.5 d            |
| 0.3                    | 4.9 b         | 35.5 d            |
| 0.6                    | 5.8 a         | 40.5 b            |
| 1.0                    | 3.5 c         | 33.2 c            |
| 1.2                    | 3.5 c         | 33.3 c            |
| 1.5                    | 3.6 c         | 32.2 c            |

**Note:** Means followed the same superscript(s) within the column are not significantly different according to LSD at $P \leq 0.05$.  

Table 1. Effect of 6-benzyl amino purine (BAP) on number of shoot formation and shoot length of *Achillea millefolium* and *Moringa peregrina* after five weeks on culture
Proteus vulgaris (10.2 mm) Klebsiella oxytoca (10.9 mm). In vitro ethanolic extracts showed Staphylococcus aureus (20.8 mm), Salmonella paratyphi (20.2 mm), Proteus mirabilis (19.8 mm), Proteus vulgaris (10.8 mm) Klebsiella oxytoca (10.8 mm). In turn, using aqueous ex vitro ethanolic extract of A. millefolium degree of inhibition zone was Salmonella paratyphi (20.7 mm), Proteus vulgaris (18.3 mm), Staphylococcus aureus (17.8 mm), Proteus mirabilis (13.9 mm), Klebsiella oxytoca (10.7 mm). Using in vitro ethanolic extract, the inhibition zone was Staphylococcus aureus (19.0 mm), Salmonella paratyphi (21.2 mm), Proteus mirabilis (14.5 mm), Proteus vulgaris (19.6 mm) Klebsiella oxytoca (14.4 mm).

Table 2. Antibacterial activity of the aqueous extract of *Moringa peregrina*, concentration 30 mg/100 µl

| Bacteria strain          | Ex vitro plants | In vitro plantlets | Positive control | Negative control |
|--------------------------|-----------------|--------------------|------------------|------------------|
| Klebsiella oxytoca       | 11.66±0.67 b    | 13.33±0.88 c       | 18.33±1.2 a      | 0.00±0.00 a      |
| ATCC 18182               |                 |                    |                  |                  |
| Proteus vulgaris         | 16.2±0.58 b     | 17.33±0.33 b       | 17.00±0.58 a     | 0.00±0.00 a      |
| ATCC 13315               |                 |                    |                  |                  |
| Proteus mirabilis        | 13.8±0.58 b     | 17.67±0.33 b       | 14.0±0.58 b      | 0.00±0.00 a      |
| ATCC 12453               |                 |                    |                  |                  |
| Staphylococcus aureus    | 19.8±0.58 a     | 22.1±0.58 a        | 17±0.58 a        | 0.00±0.00 a      |
| ATCC 25923               |                 |                    |                  |                  |
| Salmonella paratyphi     | 17.33±0.33 ab   | 20.67±0.33 a       | 14.33±0.33 b     | 0.00±0.00 a      |
| ATCC 13076               |                 |                    |                  |                  |

Note: Means followed by the same letter within the row are not significantly different according to Tukey HSD range test at P ≤ 0.05. Each treatment consisted of three replicates and each sample contained three Petri dishes. Values are the means ± standard error. Data obtained after incubation at 37 °C for 24 hours on Muller Hinton media.

Table 3. Antibacterial activity of the ethanolic extract of *Moringa peregrina* concentration 40 mg/100 µl

| Bacteria strain          | Ex vitro plants | In vitro plantlets | Positive control | Negative control |
|--------------------------|-----------------|--------------------|------------------|------------------|
| Klebsiella oxytoca       | 12.5±0.6 c      | 13.33±0.88 d       | 18.33±1.2 a      | 0.00±0.00 a      |
| ATCC 18182               |                 |                    |                  |                  |
| Proteus vulgaris         | 12.3±0.88 c     | 13.33±0.88 d       | 17.67±0.88 a     | 0.00±0.00 a      |
| ATCC 13315               |                 |                    |                  |                  |
| Proteus mirabilis        | 17.66±0.33 b    | 18.2±0.58 c        | 18.33±0.88 a     | 0.00±0.00 a      |
| ATCC 12453               |                 |                    |                  |                  |
| Staphylococcus aureus    | 22.3±0.66 a     | 23.3±0.88 a        | 17.66±0.57 a     | 0.00±0.00 a      |
| ATCC 25923               |                 |                    |                  |                  |
| Salmonella paratyphi     | 19.5±0.58 b     | 22.5±0.58 b        | 17.8±0.58 a      | 0.00±0.00 a      |
| ATCC 13076               |                 |                    |                  |                  |

Note: Means followed by the same letter within the row are not significantly different according to Tukey HSD range test at P ≤ 0.05. Each treatment consisted of three replicates and each sample contained three Petri dishes. Values are the means ± standard error. Data obtained after incubation at 37 °C for 24 hours on Muller Hinton media.

Table 4. Antibacterial activity of the aqueous extract of *Achellia millefolium*, concentration 30 mg/100 µl

| Bacteria strain          | Ex vitro plants | In vitro plantlets | Positive control | Negative control |
|--------------------------|-----------------|--------------------|------------------|------------------|
| Klebsiella oxytoca       | 10.70±0.7c      | 14.4±0.9c          | 14.5±0.9b        | 0.00±0.00 a      |
| ATCC 18182               |                 |                    |                  |                  |
| Proteus vulgaris         | 18.3±0.6b       | 19.6±0.6a          | 15.3±0.6b        | 0.00±0.00 a      |
| ATCC 13315               |                 |                    |                  |                  |
| Proteus mirabilis        | 9.2±0.6c        | 14.5±0.5c          | 17.9±0.7a        | 0.00±0.00 a      |
| ATCC 12453               |                 |                    |                  |                  |
| Staphylococcus aureus    | 17.5±0.7b       | 19.0±0.7a          | 18.5±0.8a        | 0.00±0.00 a      |
| ATCC 25923               |                 |                    |                  |                  |
| Salmonella paratyphi     | 20.7±0.5a       | 21.2±0.5 a         | 13.9±0.6 b       | 0.00±0.00 a      |
| ATCC 13076               |                 |                    |                  |                  |

Note: Means followed by the same letter within the row are not significantly different according to Tukey HSD range test at P ≤ 0.05. Each treatment consisted of three replicates and each sample contained three Petri dishes. Values are the means ± standard error. Data obtained after incubation at 37 °C for 24 hours on Muller Hinton media.
DISCUSSION

For *A. millefolium* a significant maximum number of new shoots was perceived when 1.0 mg/L was used (6.1 shoots per explants, Table 1). An increase in number of shoots was also perceived in other BA concentration compared with the control. The primary response of to the addition of BA refers to an increase in the cytosolic calcium concentration which is encouraged by its great uptake from the media (Singh et al., 2017). This performance was the same with other plant such as of *M. peregrina* (Abedllatef and Khalfall, 2010; Al Khateeb et al. 2013). Faisal et al. (2005) attained an improved response with 10.0 μM BA *Ruta graveolens*. Salem et al. (2016) reported that the effective shoot formation and multiplication of *Moringa oleifera* were obtained when nodal or shoot tip explants were cultured on MS medium supplemented with 2.5 μM BAP. This study designates that increasing concentration of BAP for *A. millefolium* enhanced the shoot formations. Moreover, BAP also contributed to a high microshoots formation in many plant types such as *Moringa peregrine* (Alrayes et al., 2016), *Achillea millefolium* (Tuker et al., 2009; Vitalini et al., 2009), *Bacopa monnieri* (Haque et al., 2017), *Ehretia microphylla* (Ranjitham et al., 2014) and *Ficus carica* (Shatnawi et al., 2019).

For *M. peregrina*, the maximum number of shoots (5.8 shoots/explants) was achieved from the explants cultivated on the medium containing 0.6 mg/L BA. Moreover, increasing BA up to 1.5 mg/L caused a significant decrease in number of shoots formed. Meanwhile, it was recorded that increased BA concentration at 0.6 mg/L significantly increased shoot length to 40.5 mm. In this concern, Atta-Alla et al. (2008) reported that higher concentration of cytokinin reduced shoot number as well as shoot length. The results in Table (1) show that various concentrations of BA assisted in the initiation *A. millefolium* (at 1.0 mg/L BA) and *M. peregrine* (at 0.6 mg/L BA) shoot proliferation. On the other hand, the highest significant length of *A. millefolium* shoots (at 0.6 mg/L BA; length of shoot 52.9) and BA at 0.6 mg/L for *M. peregrine* at 0.6 mg/L produced the shoot length (40.5). Further, a rise in the BA did not influence the shoot number. Atta-Alla et al. (2008) reported similar effects on *Ruta graveolens*. These results approved that different plant species have sufficient amount of endogenous hormones and did not have high quantity of exogenous hormone for shoot proliferation (Wala and Jasrai, 2003).

The emergence and spread of drug-resistant pathogens has made conventional antimicrobial treatments largely ineffective. Therefore, the use of traditional medicine has improved and achieves greater acceptance. This increase in the demand for finding new antimicrobial materials results from their variety of biochemicals that can be used for treatments. However, the occurrence of secondary in the plant extracts is highly linked to their biological activity. Antibiotic resistance is a challenge that continues to elude the world’s health systems (Baskaran et al., 2013; Kumari et al., 2016).

The plant extracts from *A. millefolium* and *M. perigrina* against Gram bacteria were counted previously (Alrayes et al., 2016; Faiku et al., 2018; Stojanovic et al., 2005). *A. millefolium* have been reported to have antimicrobial activity (Grigore et al., 2020). In this study, both *in vitro* and *ex vitro* (field plant) plants showed the antimicrobial activity which was superior in field

| Bacteria strain                        | Ex vitro plants | In vitro plantlets | Positive control | Negative control |
|---------------------------------------|-----------------|--------------------|------------------|------------------|
| *Klebsiella oxytoca* ATCC 18182       | 9.9± 0.6c       | 10.3± 0.9b         | 19.7± 0.9a       | 0.00± 0.00a      |
| *Proteus vulgaris* ATCC 13315         | 10.2± 0.9c      | 10.3± 0.9b         | 18.6± 0.9a       | 0.00± 0.00a      |
| *Proteus mirabilis* ATCC 12453        | 13.9± 0.5b      | 19.8± 0.8a         | 19.5± 0.7a       | 0.00± 0.00a      |
| *Staphylococcus aureus* ATCC 25923    | 19.8±0.9a       | 20.8±0.8a          | 18.3± 0.6a       | 0.00± 0.00a      |
| *Salmonella paratyphi* ATCC 13076     | 18.8±0.6a       | 20.2±0.6a          | 18.5± 0.9a       | 0.00± 0.00a      |

Note: Means followed by the same letter within the row are not significantly different according to Tukey HSD range test at P ≤ 0.05. Each treatment consisted of three replicates and each sample contained three Petri dishes. Values are the means ± standard error. Data were obtained after incubation at 37 °C for 24 hours on Muller Hinton media.
grown plants than in vitro plants (Table 2, 3, 4 and 5). Karthika et al. (2014) and Ranjitham et al. (2014) used a similar method in their studies. Some researchers reported the in vitro plants displayed higher antimicrobial activity as related to field plants (Karthika et al., 2014; Ranjitham et al., 2014). Most of them reported that field plants showed higher antimicrobial activity than in vitro plants (Karthika et al., 2014; Ranjitham et al., 2014). Thus A. millefolium and M. perigrina shown the existence of medicinally active ingredients such as alkaloids, amino acids, carbohydrates, phenolic compounds, terpenoids, steroids, proteins, saponins, coumarins, ascorbic acid and tannins as a major phytoconstituents and possessed essential antimicrobial activity (Alvarenga et al., 2018). However, several previous reports showed that the ethanol extract showed higher antimicrobial activity against different bacteria strains (Alrayes et al., 2016; Obeidat et al., 2011; Trakulsomboon et al., 2006; Abdelsalam et al., 2007). Aqueous leaves extract showed the maximum activity against Staphylococcus aureus (19.8; ex vitro plant; 22.1 in vitro plants) and Salmonella paratyphi (17.3; ex vitro plant; 20.6 in vitro plant). Therefore, this may be due to bacteria cell causing leakage of proteins and certain enzymes from their cell. The in vitro plants, generating secondary metabolites, offer a better chance for quick invention of pharmacologically significant biochemical employ for medication objectives. The result presented the potential antibacterial activities of A. millefolium and M. perigrina extracts against bacterial strains tested.

The outcomes of the current investigation obviously designate that the antimicrobial activity varies with A. millefolium and M. perigrina. These results indicate that the ex vitro and in vitro extracts of A. millefolium and M. perigrina contain the compounds with antimicrobial activity (Candan et al., 2010; Mnandhar et al., 2019). Alvarenga et al. (2018) reported that 32 volatile compounds were identified on A. millefolium, and the major ingredients were sabinene, 1.8-cineole, borneol, β-caryophyllene and β-cubebene. A. millefolium and M. perigrina could serve as valuable bases for novel antimicrobial agents. In this study, the secondary metabolites from the plant extracts have not been identified yet. Moreover, the in vitro plants show lower antimicrobial impact correlated to the ex vitro plants. The results from the study suggest that these two plants showed good antimicrobial activity against the different tested bacteria. The existing manuscript designated a very trustworthy method for large measure reproduction and the tried extracts have the possibility to hinder bacteria.

REFERENCES

1. Abdellatif, E. & Khalaftallah, M. 2010. In vitro morphogenesis studies on Moringa oleifera L. An important medicinal tree. International Journal of Biological and Medical Research, 1, 85-88.
2. Abdelsalam, H., Bogdadi, A., Kokoska, L., Havlik, J., Kloucek, P., Radav, R. & Vorisek K. 2007. In vitro antimicrobial activity of some Libyan medicinal plant extracts. Pharmaceutical Biology, 45, 386-391.
3. Al Khateeb, W., Bahar, E., Lahham, J., Schroedr, D. & Hussein, E. 2013. Regeneration and assessment of genetic fidelity of the endangered tree Moringa perigrina (Forsk.) Fiori using Inter Simple Sequence Repeat (ISSR). Physiology and Molecular Biology of Plants, 19(1), 157-164.
4. Al-Issa, R.M.S., Odat, N., Ounflye, I., Hasan, M., & Al-Tawaha, A.R. 2020. The impact of NaCl on different genotypes of tomato (Solanum Lycopersicon Mil) on germination, some physiology characteristics and gene expression. Eurasian Journal of Biosciences, 14(2), 4467-4470.
5. Alrayes, L.M.H., Al Khateeb, W.M & Shatnawi, M.A. 2016. Clonal propagation and antibacterial activity of Moringa perigrina (Forskk) Fiori plant. Journal of Advances in Biotechnology, 6(1), 787-797.
6. Alvarenga, I.C.A., Fernanda, V., Pacheco, F.V., Alvarenga, A.A., Bertolucci, S.K.Y., Jose E. & Pintoll B. P. 2018. Growth and production of volatile compounds of yarrow (Achillea millefolium L.) under different irrigation depths. Annals of the Brazilian Academy of Sciences, 90(4), 3901-3910.
7. Attal-Alla, H.K., Abo Esoud, I.H & Eid, M.H. 2008. In vitro Culture of Ruta graveolens L. Proceeding of the third international conferences on future trends in genetics and biotechnology for safe environment, Ismailia, Egypt. July 8-9, 3(3), 33-36.
8. Baskaran, P., Singh, S. & Van Staden, J. 2013. In vitro propagation, procissiilardin A production and antibacterial activity in Drimia robusta. Plant Cell Tissue and Organ Culture, 14, 259-67.
9. Bollana J.B.P., Paredes, G.E.D., Marcelo, L.W. & Idropg, C.J. 2020. In vitro tissue culture, preliminary phytochemical analysis, and antibacterial activity of Psittacanthus linearis (Killip) J.K. Macbride (Loranthaceae). Revista Colombiana de Biotecnologia, 21(2), 22-35.
10. Candan, F., Unlu, M., Tepe, B., Daferera, D., Poliissiou, M., Sokmenc, A. & Akupulata H.A. 2010.
Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). Journal of Ethnopharmacology, 87, 215-220.

11. Faiku, F., Hazori, A., Mehmeti, I., Mehmeti E, A. & Hotil, G. 2018. *In vitro* antibacterial activity of different solvent extracts of *Achillea millefolium* (L.) growing wild in Kosovo. Fresenius Environmental Bulletin, 27, 3878-3883.

12. Faisal, M., Anis, M. & Aref, M. 2010. *In vitro* calculus induction and plant regeneration from leaf explants of *Ruta graveolens* L. South African Journal of Botany, 76, 597-600.

13. Grigore, A., Colceru, M.S., Bazdoaca, C., Yulsel, R., Ionita, C. & Glava, L. 2020. Antimicrobial Activity of an *Achillea millefolium* L. Proceeding, 57 (34), 1-3.

14. Hauque, S.K.M., Chakraborty, A., Dey D., Mukherjee S., Nayak, S. & Ghosh, B. 2017. Improved micropropagation of *Bacopa monnieri* (L.) Wettst. (Plantaginaceae) and antimicrobial activity of *in vitro* and *ex vitro* raised plants against multidrug-resistant clinical isolates of urinary tract infecting (UTI) and respiratory tract infecting (RTI) bacteria. Clinical Phytoscience, 3(17), 2-10.

15. Islam, S., Jahan, M. & Khatun, R. 2005. *In vitro* regeneration and multiplication of year-round fruit bearing *Moringa oleifera*. Journal of Biological Science, 5, 145-148.

16. Karthika, K., Thnmoshi, K., Paulsmy, S. & Manian, S. 2014. Quantification of phytochemicals and *in vitro* antioxidant potential of various solvent extracts of certain species of Acanthaceae. International Journal of Green Pharmacy, 8(1), 58-64.

17. Kumari, A, Baskaran, P. & Van Staden J. 2016. *In vitro* propagation and antibacterial activity in *Cotyledon orbiculata*: a valuable medicinal plant. Plant Cell Tissue and Organ Culture, 124, 97-104.

18. Murashige T. & Skoof F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plantarum, 15, 473-497.

19. Obeidat, M., Shatnawi, M., AL-Alawi, M., AL-Zu’bi, E., Al-Dmoor, H., Ali-Qudah M., Eli-Qudah & Otri, I. 2011. Antimicrobial activity of Crude extracts of some plant leaves. Research Journal of Microbiology, 7(1), 59-67.

20. Odat, N. 2020. Intraspecific genetic variation within and between improved cultivars and landraces of durum wheat in germination and root architectural traits under saline condiation. International Journal of Plant Biology, 11(1), 13-18.

21. Oriabi A.G. 2016. *Moringa oleifera in vitro* culture and its application as anti-diabetic in alloxan induced diabetic albino mice. International Journal of Current Microbiology and Applied Sciences, 5(2), 43-49.

22. Ranjitham, P., Jamuna, S., Padmavathi, K. & Paulsamy, S. 2014. *In vitro* antioxidant properties of the traditional medicinal plant species, *Ehretia microphylla* Lam. and *Erythroxylon monogynum* Roxb. Research Journal of Pharmaceutical, Biological and Chemical Science, 5(1), 247-52.

23. Salem, J.M. 2016. *In vitro* propagation of *Moringa oleifera* L under salinity and ventilation condition. Genetics and Plant Physiology, 6(1-2), 54-64.

24. Shatnawi, M.A. 2011. Multiplication and cryogenic storage of *Artemisia herba-alba*: A medicinal plant. Journal of Food, Agriculture and Environmental, 9, 340-344.

25. Shatnawi, M.A. 2013. Multiplication and cryopreservation of Yarrow (*Achillea millefolium* L., Asteraceae). Journal of Agriculture Science and Technology, 15, 163-173.

26. Shatnawi, M.A., Shibili, R.A., Shahrour, W.G., AL-Qudah, T.S. & Abu-Zahra, T. 2019. Micropropagation and conservation of Fig (*Ficus carica* L.). Journal of Advances in Agriculture, 10, 1669-1679.

27. SPSS. 2007. Complex samples, SPSS INC., Chicago ILL: USA.

28. Stephenson, K.K. & Fahey J.W. 2004. Development of tissue culture methods for the rescue and propagation of endangered *Moringa* spp. *Germplasm*. Economic Botany, 58, 116-1-24.

29. Stojanvic, G., Radulovic, N., Hashimoto, T. & Palic, R. 2005. *In vitro* antimicrobial activity of extracts of four *Achillea* species: The composition of *Achillea clavennae* L. (Asteraceae) extract. Journal of Ethnopharmacology, 101, 185-190.

30. Trakulsomboon, S., Kummalue, T & Jiratcharakul, W. 2006. Antibacterial activities of four Thai medicinal plants. Journal of Medical Association Thailand, 89(9), 1466-71.

31. Turker A.U., Yucesan, B. & Gurel, E. 2009. *In vitro* regeneration of *Achillea millefolium* L. from shoot tips and root segments of seedlings. Journal of Plant Biochemistry and Biotechnology, 18, 65-69.

32. Vitalini S., Beretta G., Iriti M., Orsengo, S., Basilico, N., Dall’acqua S., Iorizzi M. & Filco, G. 2011. Phenolic compounds from *Achillea millefolium* L. and their bioactivity. Acta Biochimica Polonica, 58, 203-212.

33. Wala, B. & Jasrai, Y. 2003. Micropropagation of an endangered medicinal plant: *Curculigo orchioides* Gaertn. Plant Tissue Culture, 13(1), 13-19.