JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2021, 22(6), 151–158
https://doi.org/10.12911/22998993/137679
ISSN 2299-8993, License CC-BY 4.0

Received: 2021.03.30
Accepted: 2021.05.14
Published: 2021.06.01

Antimicrobial Activity and Micropropagation of Some Rare and Endangered Plants of Jordan

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ABSTRACT

The medicinal plants of Jordan are under threat due to several factors of habitat perturbation. Some of these factors include ruinous over-harvesting, climate change, competition and invasion. In this study, the authors employed a reliable method for micropropagation and assessment of antimicrobial activity of some important medicinal plants of Jordan. Seeds were surface sterilized and germinated on agar water media, and then shoots were cultured on Murashige and Skoog (MS) medium with the addition of 3% sucrose. In order to generate enough plant material, microshoots were transferred after fourteen days onto hormone free MS medium. Among all studied plants, it was found that the *Achellia millefolium* and *Moring perigrina* plants successfully multiplied *in vitro* containing different 6-benzyl amino purine (BA). For potential antimicrobial activity, the *ex vitro* (field leaf plants) and *in vitro* (plantlet) extracts were evaluated against some bacteria strains using ethanolic extracts. Both *ex vitro* and *in vitro* plants extracts showed the antimicrobial activity against the microorganism tested. The results from the study suggest that these two plants showed good antimicrobial activity against the different tested bacteria.

Keywords: medicinal plant; microbial activity; multiplication; root formation

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 121ºC 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 parattphi* ATCC 13076, *Staphcoccus 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. peregrine* 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),

Table 1. Effect of 6-benzyl amino purine (BAP) on number of shoot formation and shoot length of Achillea *millefolium* and *Moringa peregrine* after five weeks on culture

Growth regulator (mg/L)	Shoot number	Shoot length (mm)
Achillea millefollium		
0.0	1.90 e	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 peregrine		
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 2. Antibacterial activity of the aqueous extract of *Moringa peregrina*, concentration 30 mg/100 µl

Bacteria strain	Inhibition zone in (mm) on Muller Hinton plate			
	Ex vitro plants	*In vitro* plantlets	Positive control	Negative control
Klebsiella oxytoca ATCC 18182	11.66± 0.67 b	13.33± 0.88 c	18.33± 1.2 a	0.00± 0.00 a
Proteus vulgaris ATCC 13315	16.2±0.58 b	17.33± 0.33 b	17.00± 0.58 a	0.00± 0.00 a
Proteus mirabilis ATCC 12453	13.8± 0.58 b	17.67± 0.33 b	14.0± 0.58 b	0.00± 0.00 a
Staphylococcus aureus ATCC 25923	19.8± 0.58 a	22.1± 0.58 a	17± 0.58 a	0.00± 0.00 a
Salmonella paratyphi ATCC 13076	17.33± 0.33 ab	20.67± 0.33 a	14.33± 0.33 b	0.00± 0.00 a

Note: Means followed by the same letter within the row are not significantly different according to Tukey HSD range test at $P \leq 0.05$. 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	Inhibition zone in (mm) on Muller Hinton plate			
	Ex vitro plants	*In vitro* plantlets	Positive control	Negative control
Klebsiella oxytoca ATCC 18182	12.5± 0.6 c	13.33± 0.88 d	18.33± 1.2 a	0.00± 0.00 a
Proteus vulgaris ATCC 13315	12.33± 0.88 c	13.33± 0.88 d	17.67± 0.88 a	0.00± 0.00 a
Proteus mirabilis ATCC 12453	17.66± 0.33 b	18.2± 0.58 c	18.33± 0.88 a	0.00± 0.00 a
Staphylococcus aureus ATCC 25923	22.33±0.66 a	23.33±0.88 a	17.66± 0.57 a	0.00± 0.00 a
Salmonella paratyphi ATCC 13076	19.5± 0.58 b	22.5± 0.58 b	17.8± 0.58 a	0.00± 0.00 a

Note: Means followed by the same letter within the row are not significantly different according to Tukey HSD range test at $P \leq 0.05$. 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	Inhibition zone in (mm) on Muller Hinton plate			
	Ex vitro plants	*In vitro* plantlets	Positive control	Negative control
Klebsiella oxytoca ATCC 18182	10.70± 0.7c	14.4± 0.9c	14.5± 0.9b	0.00± 0.00 a
Proteus vulgaris ATCC 13315	18.3±0.6b	19.6± 0.6a	15.3± 0.6b	0.00± 0.00 a
Proteus mirabilis ATCC 12453	9.2± 0.6c	14.5± 0.5c	17.9± 0.7a	0.00± 0.00 a
Staphylococcus aureus ATCC 25923	17.5± 0.7b	19.0± 0.7a	18.5± 0.8a	0.00± 0.00 a
Salmonella paratyphi ATCC 13076	20.7± 0.5a	21.2± 0.5 a	13.9± 0.6 b	0.00± 0.00 a

Note: Means followed by the same letter within the row are not significantly different according to Tukey HSD range test at $P \leq 0.05$. 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.

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 5. Antibacterial activity of the ethanolic extract of *Achellia millefolium* concentration 40 mg/100 µl

Bacteria strain	Inhibition zone in (mm) on Muller Hinton plate			
	Ex vitro plants	*In vitro* plantlets	Positive control	Negative control
Klebsiella oxytoca ATCC 18182	9.9± 0.6c	10.33± 0.9b	19.7± 0.9a	0.00± 0.00a
Proteus vulgaris ATCC 13315	10.2± 0.9c	10.33± 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± 0.00 a
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 $P \leq 0.05$. 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.

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 gravelones*. 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

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. peregrina*. These results indicate that the *ex vitro* and *in vitro* extracts of *A. millefolium* and *M. peregrina* 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. millefoulim*, and the major ingredients were sabinene, 1.8-cineole, borneol, β-caryophyllene and β-cubebene. *A. millefolium* and *M. peregrina* 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.

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