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Clonal Propagation and Antibacterial Activity of Moringa Peregrina (Forssk) Fiori Plant

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ABSTRACT

Moringa peregrina (Forssk) Fiori is one of the known medicinal plants in Jordan. It is used in traditional medicine to treat rheumatism and infections. Plant parts are used in the indigenous systems of human medicine for the treatment of a variety of ailments. Therefore, this study was conducted to investigate factors affecting *M. peregrina in vitro* propagation and its antimicrobial activity. Microshoots with apical meristem (10 or 15 mm in length) were subculture on MS medium supplemented with different concentration of BAP, Kinetin, Zeatin and Thidiazuron (TDZ). Maximum number of new shoots/explant (4.39) was obtained on Murashige and Skoog agar medium supplemented with 1.6 mg/L Zeatin. While at 0.4 mg/L BAP maximum shoot length was obtained (37.78 mm). Antibacterial activity of aqueous, methanolic, ethanolic, and acetonic extracts of both *in vitro* plantlets and *ex vitro* (field grown) *M. peregrina* were evaluated by the agar well-diffusion method against *Klebsiella oxytoca* ATCC 18182, *Salmonella typhimurium* ATCC 19430, *Methicillin resistant, Staphylococcus aureus* ATCC 29974, *Klebsiella pneumonia* ATCC13883, *Proteus vulgaris* ATCC 13315, *Proteus mirabilis* ATCC 12453, *Enterobacter aerogenes* ATCC 35029, *Pseudomonas aeruginosa* ATCC 27253, *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 25923, *Salmonella paratyphi* ATCC 13076 and *Escherichia coli* ATCC 29522. The obtained results showed that ethanolic, acetonic and aqueous extracts revealed a wide antibacterial activity. Ethanolic extract of *in vitro M. peregrina* plantlets showed the maximum inhibition zone against *Staphylococcus aureus*.

Indexing terms/Keywords

Antibacterial; aqueous extract; *in vitro*; micrporpagation; *Moringa peregrina*.

Academic Discipline And Sub-Disciplines

Biotechnology

SUBJECT CLASSIFICATION

Plant tissue culture, Microbiology.

TYPE (METHOD/APPROACH)

Expirement.

INTRODUCTION

Medicinal plants are considered as an important source of medication for thousands of years. The Egyptian pharmacopoeia of Ebers Papyrus described the uses of medicinal plants like opium and castor oil (Gossell-Williams *et al*., 2006). Nowadays, there is a growing interest for using medicinal plants as natural sources in pharmaceutical and food industries (Ahmad and Beg, 2001). According to the World Health Organization (WHO) about 80% of the world's population mainly in some Asian and African countries and less in developed countries depends on herbal medicine for their care (WHO, 2008). Moreover, medicinal plants have been used for antihypertensive, anti-asthmatic, anti-diabetic, antimicrobial activities for centuries (Kaçar, 2008).

In Jordan, medicinal plants constitute an important component of Jordanian flora because of its geographic location, climate, and the presence of nearly 2,500 natural plant species. More than 500 species are classified as medicinal plants which are widely distributed all over the country and used for the treatment of various diseases (Al-Eisawi, 1982; Oran and Al-Eisawi, 1998; Afifi and Abu-Irmaileh, 2003). *Moring peregrina* is highly nutritious, which contains more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges and more potassium than bananas and more protein than milk and eggs (Sreelatha *et al*., 2011). Moreover, leaves of *M. peregrina* plants rich in various phytochemicals such as carotenoids, amino acids, sterols, glycosides, alkaloids, flavonoids, moringine, moringinine, phytoestrogens caffeoylquinic acids and phenolics (Anwar *et al*., 2007). Also, roots, flowers, gum and seeds are extensively used for treating inflammation, cardiovascular action, liver diseases, hematological, hepatic and renal function (Mazumder *et al*., 1999).

M. peregrina in Jordan becoming an endangered plant as a result of unmanaged grazing and slow regeneration rate after browsing (Gomma and Pico, 2011; Steinitz *et al*., 2009). *M. peregrina* occurs naturally in lower Jordan valley, Dead Sea area, Wadi Araba and Wadi Feynan (Al Kahtani and Abu Arab, 1993). The conventional propagation methods of *M. peregrina* by seeds and cutting are not preferred due to low germination percentage and rooting problems (Abd El Baky and El-Baroty, 2013). *In vitro* culture of *M. peregrina* can solve propagation problems; it guarantees mass production of plant material without menacing natural resources and also it improves and conserves this plant (Al Khateeb *et al*., 2013). Therefore, the aims of this study were to develop clonal propagation for *M. peregrina* plant as well as to evaluate the antibacterial activities of *in vitro* plantlets and *ex vitro* plants.

MATERIALS AND METHODS

Effect of cytokinins on shoot proliferation

Tissue culture plants were obtained from Yarmouk University, which were established previously at plant genetic and biotechnology laboratory. Wild plants were obtained from South of Dead Sea/ Jordan (31 °74′880″N; 35 °59′378″E). Microshoots were subcultured on Murashige and Skoog medium(1962) supplemented with 0.5 mM myo-inositol, 0.34 mM thiamine HCl, 2.4 mM pyridoxine HCl, 4.1 mM nicotinic acid and 3% sucrose, without growth regulators and incubated for four week at 24 ± 2 ºC with a 16/8 h light/ dark. Photoperiod and photosynthetic photon flux density (PPFD) of 50 µmol m-^{2s}-1 supplied by cool white florescent lamps. For shoot proliferation, the produced microshoots were subcultured on Murashige and Skoog (MS) medium supplemented with different concentration of BAP, Kinetin, Zeatin and Thidiazuron (TDZ). Each treatment consisted of 18 replicates and each replicate contained three microshoots. Microshoots were incubated at 24 ± 2 ºC with a 16/8 h light/ dark. Photoperiod and photosynthetic photon flux density (PPFD) of 50 µmol m^{-2} s^{-1} supplied by cool white florescent lamps. Data were collected after six weeks including the number of newly formed shoots, maximum shoot length and the number of leaves per explant.

Antibacterial activity

Antibacterial activities of different *M. peregrina* plants extracts were evaluated by the agar well-diffusion method and minimum inhibitory concentration (MIC) according to Akinyemi *et al*. (2005) method.

Preparation and its sterilization

Antibacterial susceptibility was tested on solid media in petri plates, 38 gm of Muller Hinton Agar was added to 1000 ml of sterile distilled water and autoclaved at 121ºC for 15 minutes after cooling the media (about 15 ml) were poured to 9 cm sterile Petri dishes to obtain 0.4 cm thickness. Then 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.

Preparation of plant extract

M. peregrina plants materials (*ex vitro*; field grown plants) and *in vitro* plantlets and callus) were dried in the shade for two weeks, ground to a fine powder by using a blender. 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 grinded 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), stored in sterile bottles and kept in refrigerator then evaluated for their ability to inhibit the bacterial. 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

Test organisms

Twelve bacterial species were tested as follow: *Klebsiella oxytoca* ATCC 18182, *Salmonella typhimurium* ATCC 19430,*Methicillin resistant Staphylococcus aureus* ATCC 29974, *Klebsiella pneumonia* ATCC13883, *Proteus vulgaris* ATCC 13315, *Proteus mirabilis* ATCC 12453, *Enterobacter aerogenes* ATCC 35029, *Pseudomonas aeruginosa* ATCC 27253, *Escherichia coli* O157:H7, *Staphylococcus aureus* ATCC 25923, *Salmonella paratyphi* ATCC 13076 and *Escherichia coli* ATCC 29522. Bacterial cultures were obtained from the laboratory of Biotechnology at Al-Balqa Applied University, Jordan. The pure bacterial cultures were maintained on nutrient broth medium at 4°C.

For determination of antibacterial activity, bacterial cultures were adjusted to 0.5 McFarland turbidity standards (Ataee *et al*., 2012). Within 15 minutes after adjusting the turbidity of the inoculums suspension this basic suspension should contain approximately 10^7 to 10^8 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). 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 hours for bacterial pathogens. Control experiments comprising inoculate without plant extract were set up. Antimicrobial activity was determined by measuring the zone of inhibition around each well in mm at the end of the incubation period. The results were compared with the control antibiotic, kanamycin (Agwa *et al*., 2000). For each extract three replicate trials were conducted against each organism.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The plant extracts which were found effective, as antimicrobial agent, were later tested to determine the MIC and MBC values for each strain. MIC was determined using broth dilution method. The extracts were diluted to give the final concentrations of 40, 20, 10, 5, 2.5, 1.25, .652 mg/100µl. 100 µl of 10^5 CFU/ml of each strain were inoculated in tubes with equal volume of nutrient broth and plant extracts. The tubes were incubated aerobically at 37 °C for 24-48 hours. Three control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24 hrs when compared with the control tubes was considered as initial MIC. The dilutions that showed no turbidity were incubated further for another 24 hrs at 37 °C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 h was regarded as final MIC. MBC value was determined by subculturing the test dilution [which showed no visible turbidity] on to freshly prepared nutrient agar media. The plates were incubated further for 18-42 h at 37 °C. Maximum dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC.

Statistical analysis

The experiments were designed as completely randomized design. Data were subjected to ANOVA analysis. Mean values were compared according to Tukey HSD Multiple Range test at P= 0.05. Data were analyzed using SPSS (SPSS, 2010).

RESULTS

Shoot multiplication

Effect of benzyl amino purine (BAP)

Maximum number of shoots (3.17 shoot per explants) was obtained on MS supplemented with 0.8 mg/L BAP (Table 1). While at 0.4 mg/L BAP maximum shoot length was obtained (37.78 mm) compared with the control shoot length (31.83 mm), and the maximum number of leaves (14.22 leaves per explant) compared with the control number of leaves (6.67 leaves per explant) (Fig. 1). On the other hand, the maximum callus diameter was obtained on MS medium supplemented with 0.4 mg/L BAP.

Table 1. Effect of 6-benzyl amino purine (BAP) on mean number of axillary shoots per explants, mean shoot length, and mean number of leaves of *in vitro* produced *Moringa peregrina* plantlets after six weeks on culture.

BAP (mg/L)	Number of axillary shoots / explants	Shoot length (mm)	Number of leaves / explants	Callusing
0.0	1.89±0.22 a	31.83±4.58 bc	6.67±0.88 ab	+
0.4	2.83±0.25 ab	37.78±5.21 c	14.22±1.50 c	+++
0.8	3.17±0.43 b	18.17±2.54 ab	13±2.48 bc	++
1.2	2.5±0.39 ab	17.61±2.46 a	12.56±2.00 bc	++
1.6	2.11±0.21 ab	6.28±0.60 a	2.94±.54 a	-

Means followed by the same letter within the column are not significantly different according to Tukey HSD test at P≤ 0.05. Each treatment consisted of 18 replicates and each sample contained three microshoots. Values are the means ± standard error. Callus column: (–) represents no callus, (+) represents < 5 mm in diameter, (++) represents ≥ 5 mm and < 8 mm in diameter, (+++) represents ≥ 8 and <11 mm in diameter and (++++) represents ≥11 mm in diameter

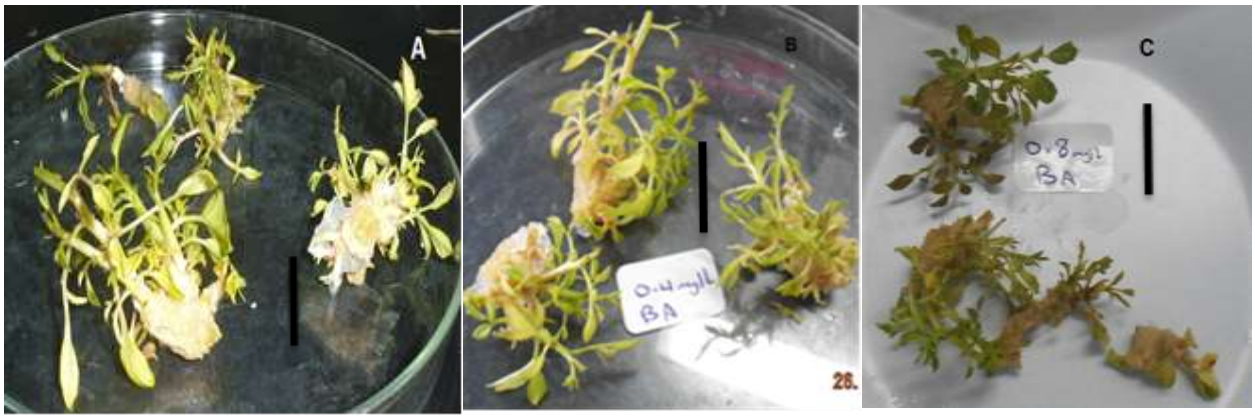

Figure 1: Formation of multiple shoots of *Moringa peregrina* grown on MS medium after six weeks growth periods. A) MS free hormone medium B) MS medium supplemented with 0.4 mg/L6-benzyl amino purine (BAP). C) MS medium supplemented with 0.8 mg/L6-benzyl amino purine (BAP). Bars represent 5 mm.

Effect of kinetin

Supplement of kinetin to MS medium produced maximum shoot length of (34.72 mm) at 1.6 mg/L compared with the control culture (31.83 mm) (Fig. 2). Increased kinetin did not show any significant difference on neither the number of new shoot per explants nor on the callus formation (Table 2).

Table 2: Effect of kinetin on mean number of axillary shoots per explant, mean shoot length and mean number of leaves of *in vitro* produced *Moringa peregrina* plantlets after six weeks on culture

Kinetin (mg/L)	Number of axillary shoots/explants	Shoot length (mm)	Number of leaves / explants	Callusing
0.0	1.89±0.22 b	31.83±4.58 a	6.67±0.88 a	+
0.4	1.5± 0.21 ab	26.39± 4.79 a	6.06± 1.41 a	+
0.8	1.33± 0.11 ab	28.06± 3.59 a	5.67± 0.99 a	-
1.2	1.83± 0.20 ab	25.89± 5.25 a	6.94± 1.33 a	+
1.6	1.06± 0.20 a	34.72± 6.67 a	6.06± 1.18 a	+

Means followed by the same letter within the column are not significantly different according to Tukey HSD test at P≤ 0.05. Each treatment consisted of 18 replicates and each sample contained three microshoots. Values are the means ± standard error. (–) represents no callus, (+) represents < 5 mm in diameter, (++) represents ≥ 5 mm and < 8 mm in diameter, (+++) represents ≥ 8 and <11 mm in diameter and (++++) represents ≥11 mm in diameter.

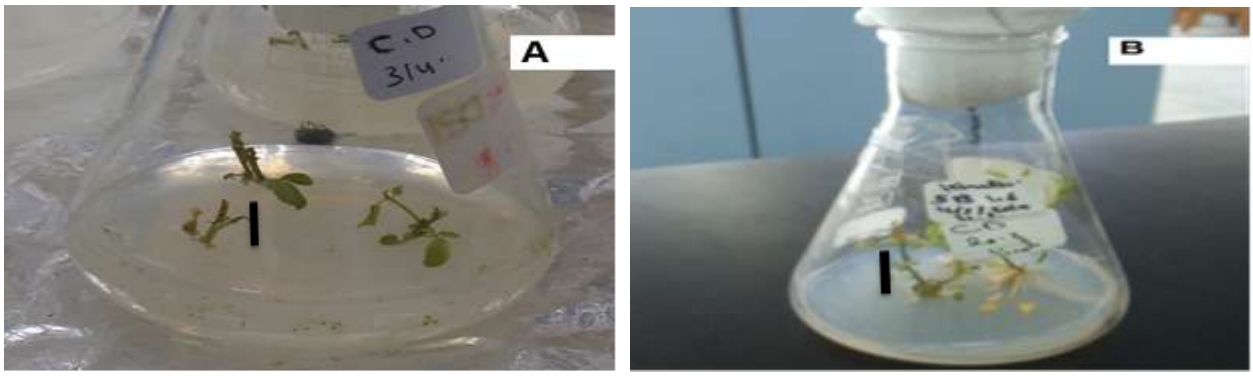

Figure 2: Formation of multiple shoots of *Moringa peregrina* grown on MS medium supplemented with 1.6 mg/L Kinetin. A). *Moringa peregrina in vitro* plantlet after two weeks growth periods. B) *Moringa peregrina in vitro* plantlet after six weeks growth periods Bars represent 5 mm.

Effect of thidiazuron (TDZ)

TDZ at a concentration of 1.6 mg/L produce 2.78 shoot per explant. While TDZ didn't have any positive effect on shoot length or number of leaves per explants (Table 3) (Fig. 3), it has a significant effect on callus induction.

Table 3: Effect of Thidiazuron (TDZ) on mean number of axillary shoots per explant mean shoot length and mean number of leaves of *in vitro* produced *Moringa peregrina* plantlets after six weeks on culture.

TDZ (mg/L)	Number of axillary shoots/explants	Shoot length (mm)	Number of leaves / explants	Callusing
0.0	1.89± 0.22 a	31.83± 4.58 b	6.67± 0.89 a	+
0.4	2.67± 0.24 ab	12.06± 1.34 a	6.44± 0.67 a	+++
0.8	2.22± 0.19 ab	13.06± 2.18 a	6.39± 0.82 a	+++
1.2	2.11± 0.20 ab	13.61± 1.56 a	6.22± 0.74 a	++++
1.6	2.78± 0.21 b	13.61± 1.56 a	7.44± 0.78 a	++++

Means followed by the same letter within the column are not significantly different according to Tukey HSD test at P≤ 0.05. Each treatment consisted of 18 replicates and each sample contained three microshoots. Values are the means ± standard error. (–) represents no callus, (+) represents < 5 mm in diameter, (++) represents ≥ 5 mm and < 8 mm in diameter, (+++) represents ≥ 8 mm and <11 mm in diameter and (++++) represents ≥ 11 mm in diameter.

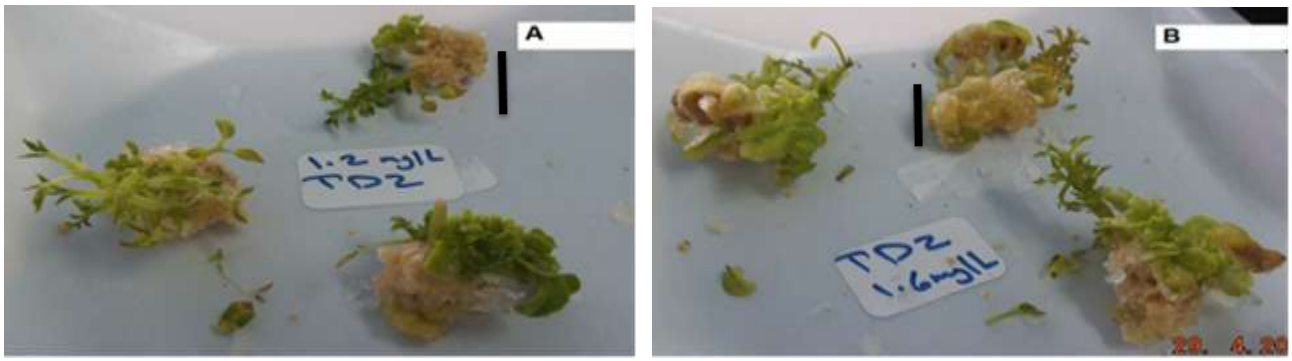

Figure 3: Formation of multiple shoots of *Moringa peregrina* grown on MS media after 6 weeks in culture. A) Multiple shoot on MS medium supplemented with 1.2 mg/L Thidiazuron (TDZ). B) Multiple shoot on MS medium supplemented with 1.6 mg/L Thidiazuron (TDZ). Bars represent 5 mm.

Effect of zeatin

Maximum numbers of shoots (4.39 shoots per explants) were obtained on MS medium supplemented with 1.6 mg/L Zeatin (Table 7). While the maximum shoot length (35.2) was recorded on MS medium supplemented with 0.8 mg/L Zeatin. Maximum number of leaves per explant (14.17 leaves per explant) was obtained on MS medium supplemented with 0.8 mg/L Zeatin (Table 4) (Fig. 4) On the other hand, increasing Zeatin concentration has increased callus induction (Table4).

Table 4: Effect of Zeatin on number of axillary shoot per explant, shoot length and number of leaves of *in vitro* produced *Moringa peregrina* plantlets after six weeks on culture.

Zeatin (mg/L)	Number of axillary shoots/explants	Shoot length (mm)	Number of leaves / explants	Callusing
0.0	1.89± 0.22 a	31.83± 4.58 a	6.67± 0.89 a	+
0.4	3.83± 0.33 b	31.38±2.71 a	11.28± 1.23 ab	++
0.8	4.00± 0.56 b	35.28±4.32 a	14.17± 1.69 b	+++
1.2	3.11± 0.38 ab	29.17± 3.46 a	11.89±1.66 ab	+++
1.6	4.39± 0.39 b	21.94± 3.21 a	13.83± 1.71 b	++++

Means followed by the same letter within the column are not significantly different according toTukey HSD test at P≤ 0.05. Each treatment consisted of 18 replicates and each sample contained three microshoots. Values are the means ±standard error. (–) represents no callus, (+) represents < 5 mm in diameter, (++) represents ≥ 5mm and < 8 mm in diameter, (+++) represents ≥ 8 and <11 mm in diameter and (++++) represents ≥ 11 mm in diameter

Figure 4: Multiple shoot formation of *Moringa peregrina* grown on MS media after one week in culture. A) Multiple shoot on MS medium supplemented with 0.8 mg/L Zeatin. B) Multiple shoot on MS medium supplemented with 1.6 mg/L Zeatin. Bars represent 5 mm.

Antibacterial activity

Plant extract (*in vitro* and *ex vitro*) of *M. peregrina* prepared in different solvent (aqueous, acetone, ethanol or methanol) was found to be more effective against gram negative bacteria than gram positive. Various bacterial isolates such as *Proteus vulgaris*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Escherichia coli* and *Salmonella typhimurium* were inhibited by the using of the different plant extracts as shown in Tables number 5, 6, 7 and 8, and Figs 5 and 6. Aqueous extract inhibited growth of *Proteus mirabilis*, *Staphylococcus aureus* and *Salmonella paratyphi* (Table 5). While *in vitro* methanolic plant extract was more effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus* than *ex vitro* extract and the control (Table 6, Fig. 6). Whereas *Staphylococcus aureus* was the most sensitive for both ethanolic and acetonic plant extracts (Tables 7 and 8).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extract using different solvents were studied. The results showed that the (MIC) and the (MBC) for all the sensitive strain of *in vitro* extracts were always less or equal to the (MIC) and (MBC) of *ex vitro* extract especially with *Staphylococcus aureus*. Both MIC and MBC of *in vitro* aqueous extract was ten folders lower than those of *ex vitro* plant extract Tables (9, 10, 11 and 12). On the contrary, *Escherichia coli* MIC and MBC of the *in vitro* acetonic extract were higher than those of *ex vitro* acetonic plant extract (Table 12).

Table 5: Antibacterial activity of *Moringa peregrina* aqueous extract, concentration 30 mg/100µl.

Strain	Inhibition zone in (mm) on Muller Hinton plate			
	***Ex vitro* Plants**	***In vitro* Plantlets**	**Positive control**	**Negative control**
Proteus vulgaris ATCC 13315	16±0.58 b	17.33± 0.33 b	17.00± 0.58 b	0.00± 0.00 a
Proteus mirabilis ATCC 12453	14± 0.58 b	17.67± 0.33 c	14.0± 0.58 b	0.00± 0.00 a
Staphylococcus aureus ATCC 25923	19± 0.58 b	22± 0.58 c	17± 0.58 b	0.00± 0.00 a
Salmonella paratyphi ATCC 13076	17.33± 0.33c	20.67± 0.33 d	15.33± 0.33 b	0.00± 0.00 a

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 6: Antibacterial activity of *Moringa peregrina* methanolic extract, concentration 40mg/100µl.

Strain	Inhibition zone in (mm) on Muller Hinton plate			
	***Ex vitro* Plants**	***In vitro* Plantlets**	**Positive control**	**Negative control**
Klebisella oxytoca ATCC 18182	12± 0.58 b	13.67± 0.67 b	18.33± 0.88 c	0.00± 0.00 a
Pseudomonas aeruginosa ATCC 27253	17.67± 0.33b	19.33± 0.88 b	17.67± 0.88 b	0.00± 0.00 a
Staphylococcus aureus ATCC 25923	19.66± 0.88b	21.33± 0.88 b	18.33± 0.88 b	0.00± 0.00 a

Means followed by the same letter within the row are not significantly different according to Tukey HSDrange 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 7: Antibacterial activity of *Moringa peregrina* ethanolic extract, concentration 40mg/100µl.

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± 0.67b	13.33± 0.88 b	18.33± 1.2 c	0.00± 0.00 a
Proteus vulgaris ATCC 13315	12.33± 0b	13.33± 0.88 b	16.67± 0.88 c	0.00± 0.00 a
Pseudomonas aeruginosa ATCC 27253	17.66± 0.33b	18± 0.58 b	18.33± 0.88 b	0.00± 0.00 a
Staphylococcus aureus ATCC 25923	24.33±0.66 c	28.33±0.88 d	17.66± 0.57 b	0.00± 0.00 a

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 8: Antibacterial activity of *Moringa peregrina* acetonic extract, concentration 40 mg/100µl.

Strain	Inhibition zone in (mm) on Muller Hinton plate			
	***Ex vitro* Plants**	***In vitro* Plantlets**	**Positive control**	**Negative control**
Klebsiella oxytoca ATCC 18182	20.33± 0.88b	21.33± 0.67 b	19± 0.58 b	0.00± 0.00 a
Salmonella typhimurium ATCC 19430	14.67± 0.88b	17.33± 1.45 b	16± 0.58 b	0.00± 0.00 a
Pseudomonas aeruginosa ATCC 27253	17± 0.58 b	19± 0.58 c	17.67± 0.33bc	0.00± 0.00 a
Salmonella paratyphi ATCC 13076	16± 0.58 b	15.33± 0.88 b	16± 0.58 b	0.00± 0.00 a
Escherichia coli ATCC 29522	14± 0.58 b	13± 0.58 b	14.33± 0.33 b	0.00± 0.00 a
Staphylococcus aureus ATCC 25923	18.33±0.33 b	27.33±1.45 c	17.66±0.57 b	0.00± 0.00 a

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.

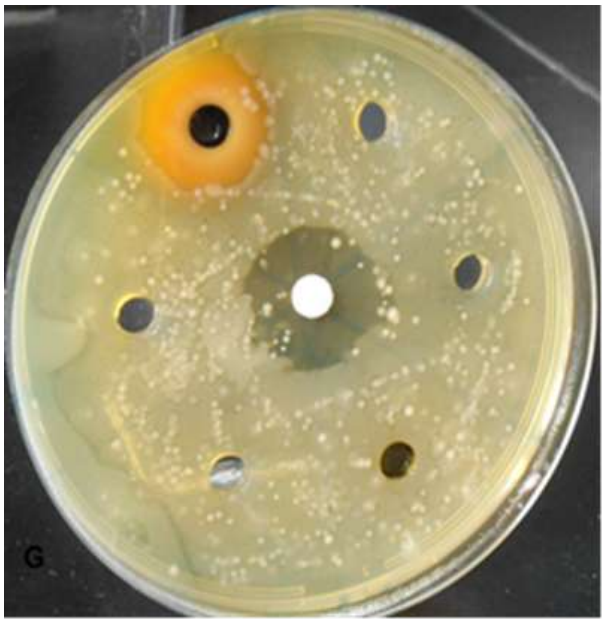

Figure 5: Comparison between *in vitro* acetonic extract and the positive control against *Klebsiella oxytoca*, the centre of the plate represents positive control

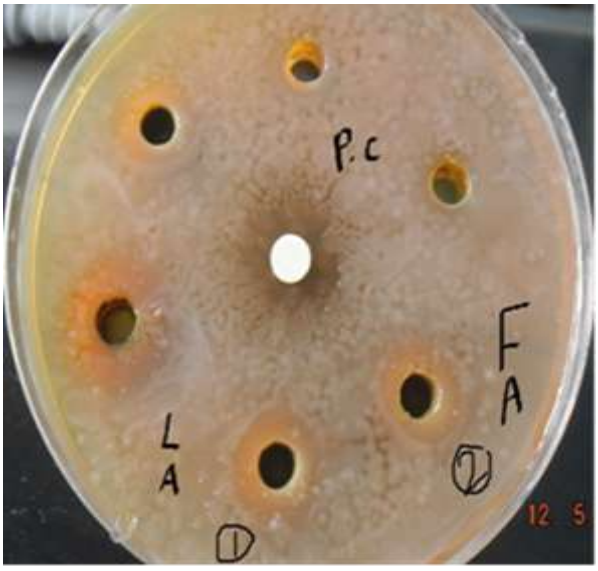

Figure 6: Antibacterial activity of *ex vitro* and *in vitro* methanolic extract and the positive control against *Pseudomonas aeruginosa* 1) Represents *in vitro* methanolic activity against the bacteria. 2) Represents *ex vitro* methanolic extract against the same bacteria. P.C) Represents the positive control.

Table 9: Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Moringa peregrina* aqueous extract mg/100µl

Strain	*Ex vitro* (MIC)	*Ex vitro* (MBC)	*In vitro* (MIC)	*In vitro* (MBC)
Proteus vulgaris ATCC 13315	7.5a	10	2.5	5
Proteus mirabilis ATCC 12453	10	12.5	10	7.5
Staphylococcus aureus ATCC 25923	10	12.5	0.625	1.25
Salmonella paratyphi ATCC 13076	12.5	15	7.5	10

a values are means of triplicate determination

Table 10: Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Moringa peregrina* methanolic extract mg/100µl

Strain	*Ex vitro* (MIC)	*Ex vitro* (MBC)	*In vitro* (MIC)	*In vitro* (MBC)
Klebsiella oxytoca ATCC 18182	5a	7.5	2.5	5
Pseudomonas aeruginosa ATCC 27253	2.5	5	2.5	5
Staphylococcus aureus ATCC 25923	2.5	5	1.25	2.5

a values are means of triplicate determination

Table 11: Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Moringa peregrina* ethanolic extract mg/100µl

Strain	*Ex vitro* (MIC)	*Ex vitro* (MBC)	*In vitro* (MIC)	*In vitro* (MBC)
Klebsiella oxytoca ATCC 18182	7.5 a	10	5	7.5
Proteus vulgaris ATCC 13315	15	17.5	10	12.5
Pseudomonas aeruginosa ATCC 27253	5	7.5	5	7.5
Staphylococcus aureus ATCC 25923	2.5	5	1.25	2.5

a values are means of triplicate determination

Table 12 : Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Moringa peregrina* acetonic extract mg/100µl

Strain	*Ex vitro* (MIC)	*Ex vitro* (MBC)	*In vitro* (MIC)	*In vitro* (MBC)
Klebsiella oxytoca ATCC 18182	2.5 a	5	1.25	2.5
Salmonella typhimurium ATCC 19430	5	7.5	2.5	5
Pseudomonas aeruginosa ATCC 27253	5	7.5	2.5	5
Staphylococcus aureus ATCC 25923	2.5	5	1.25	2.5
Salmonella paratyphi ATCC 13076	5	7.5	5	7.5
Escherichia coli ATCC 29522	2.5	5	5	7.5

a values are means of triplicate determination

RESULTS

BAP, TDZ, Zeatin and kinetin produced small multiple shoots, while kinetin did not increased the number of new formed shoot significantly (Tables 1-4). Increased BAP up to 0.8 mg/L showed maximum formation of micorshoots, whereas at 1.6 mg/ L BAP, the formation of new shoots decreased significantly (Table 1). A study on *M. peregrina* showed that optimum shoot proliferation was obtained on MS medium containing 1.0 mg/L BAP (Al Khateeb *et al*., 2013). Whereas, proliferation was decreased at 2.0 mg/L BAP, this is similar to our finding on Table (1). These results are also close to those which were obtained by Islam *et al*. (2005), who reported a high percent rates shoot induction on *Moringa oleifera* nodes using BAP at 1.0 and 1.5 mg/L. On the other hand, Riyathong *et al*. (2012) showed that 100% shoot formation was obtained by using MS medium supplemented with BA at 2.0 mg/L. Also Karim *et al*., (2002) indicated that 1.0 mg/L BAP produced maximum shoot initiation in chrysanthemum. Levels of 1.0 mg/L BAP and above induced toxicity to the explants, and thus would be unsuitable for micropropagation. Conversely, kinetin did not show improvement on *in vitro* growth. Media supplemented with different kinetin concentration did not enhance shoot proliferation of *in vitro* culture of *M. peregrina*.

Of the fourth cytokinins tried, Zeatin proved to be better than BAP, kinetin or TDZ for shoot induction from shoot apical meristem. Zeatin at 1.6 mg/L produced maximum number of shoots. This may be due to the suppression of apical

dominance that leads to production of more number of multiple shoots and reduced shoot length. Similar to our result, Wala and Jasrai. (2003) reported that maximum number shoots of *Curculigo orchioides* were obtained with a low level of BA (2.21 µM). Further increase in the concentration of BA had no effect on the number of multiple shoots. In contrast, Shibli *et al*. (1998) reported that increasing BA concentrations (1.5-2 mg/L) increased the number of shoots, but decreased the shoot length for in vitro propagated apple root stock (MM 106). Moreover, Purohit *et al*. (1994) found that genus of a (Liliaceae) family, Chlorophytum, required a relatively high concentration of BA (22.2 µM) for shoot regeneration.These results confirmed that some plant species have enough levels of endogenous hormones and does not require high levels of exogenous growth regulators for plant growth (Pierik 1997).

In the current study, preliminary screening for antibacterial activities showed, that the aqueous, ethanolic, acetonic and methanolic extracts of *M. peregrina* exhibited inhibitory effect against some bacterial species. The antimicrobial assay by agar-well diffusion method revealed that methanol extract, ethanolic, acetonic as well aqueous *M. peregrina* extracts exhibited broad spectrum activity against tested isolates (Tables 5, 6, 7 and 8). Thus, the study ascertains the value of *M. peregrina* plants used in disease treatment, which could be of considerable interest to the development of new drugs. Our preliminary investigation showed that ethanol, methanol, acetone and aqueous extracts of *M. peregrina* were active against *Proteus vulgaris*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Escherichia coli* and *Salmonella typhimurium* (Tables 5, 6, 7 and 8). *Ex vitro*, *in vitro* plantlets and callus extracts of *M. peregrina* demonstrated a broad-spectrum activity against gram-negative bacteria more than gram-positive, this possibly due to the presence of many chemicals in *M. peregrina* plant.

CONCLUSION

The result of the present investigation indicated that an efficient *in vitro* propagation method could be achieved for *Moringa peregrina*. Furthermore, *ex vitro* (wild grown) and *in vitro M. peregrina* extracts showed high antibacterial activity against a large number of pathogenic bacteria.,

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