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Full Length Article

Antimicrobial Activity and Micropropagation of *Ruta graveolens* Medicinal Plant

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Abstract

The present study was conducted to develop protocol for plant propagation and antimicrobial examination of *Ruta graveolens* L. through *in vitro* shoot. Murashige and Skoog (MS) medium contain different concentrations of thidiazuron (TDZ) and Kinetine**.** The Maximum of new microshoots number (9.44) was obtained using 1.5 mg/L TDZ. Using TDZ at 1.5 mg/L produces the maximum shoot number of *R. graveolens*. *In vitro* and *ex vitro* leaf extracts were screened against some bacteria and fungi using crude extracts. The crude leaf extract from *in vitro* and *ex vitro R. graveolens* dissolved in various solvent solutions showed different activity against both some bacteria and fungi. The methanolic and the ethanolic extracts were affectional against bacteria and fungi. *In vitro* ethanolic extract was discovered to be non-affectional for some fungi including *Penicillium chrysogenum*, *P. italicum*, and *Aspergillus nidulans*, On the other hand, methanolic extract was not affectional against *P. chrysogenum*. Maximum inhibition with ethanolic extract was found to be affectional against *Candida albicans*, *P. digitatum* and *P. italicum*. While methanolic was found to be affectional against *C. albicans*, *P. digitatum* and *P. italicum*. This study shows that methanolic and ethanolic used affects the antibacterial and antifungal activity. Moreover, *R. graveolens* could be used in the pharmaceutical industry as an ordinary source for antibacterial and antifungal treatments. © 2022 Friends Science Publishers

Keywords: Antimicrobial; *Candida albican;* Medicinal plant; Micropropagation*; Penicillium chrysogenu; Ruta graveolens*

Introduction

Medicinal plants have an important medicinal plant for the well-being of people and beneficiation to the economy (Alrayes *et al*. 2018; Shhab *et al*. 2021). *Ruta graveolens* L. belongs to the Rutaceae family. It is an odorous aromatic medicinal plant. Rutaceae family is a perennial plant that consists of many types of secondary metabolites, such as furanocoumarins, alkaloids, flavonoids, alkaloids, and essential oils (Diwan and Malpathak 2007; Kengar and Paratkar 2015; Mahmoud *et al*. 2015). *R. graveolens* known as rue has been used in traditional medicine. *R. graveolens* is used for the remedy of various treatments such as eye problems, rheumatism, dermatitis, psoriasis, vitiligo, and leucoderma pain (Retheesh and Helen 2007; Diwan *et al.* 2012; Al-Ajlouni *et al.* 2015; Orlanda and Nascimento 2015; Hadi *et al*. 2019).

The germination percentage of *R. graveolens* seeds was low and the seed set also is low does not allow the production of the true-to-type plant resulting in a great variant of secondary metabolites (Faisal and Naseem 2005; Orlanda and Nascimento 2015; Hadi *et al*. 2019). In addition, *R. graveolens* obtained shoots would not be genetically identical to the parent plant and the genetic makeup may vary with the individual shoots because this plant is a cross-pollinated plant. Due to the above problems, *in vitro* propagation method is the only option for high propagation grade of genetically uniform *R. graveolens* plants. Many previous studies have been successfully propagated using *in vitro* propagation methods (Kunicka-Styczy ´nska and Gibka 2010; Parray *et al*. 2012; Reddy *et al*. 2015; Shatnawi *et al.* 2019; González-Locarno *et al.* 2020). *In vitro* propagation techniques may assist in the propagation and preserve endangered and rare medicinal plants. The media compositions and plant growth regulators play a vital role in *in vitro* propagation of plants. Therefore, there is an urgent need to look for alternate means of propagation for *R. graveolens* which could ensure high mass-producing plants to fulfill the demands of these plants (Atta-Alla *et al*. 2008; Parray *et al.* 2012; Shhab *et al*.

2021). *In vitro* propagated medicinal plants could offer a ready source of even, sterile, and compatible plant material for biochemical characterization and identification of bioactive constituents (Shhab *et al.* 2021).

R. graveolens contain pharmaceutical active compounds such as alkaloids, coumarins, volatile oils, and flavonoids that have antimicrobial activity have antimicrobial activity. The antimicrobial activity of *R. graveolens* has been studied by Reddey and Al-Rajab (2016) and Parray *et al*. (2012). *R. graveolens* has the capability to forbid the growth of some microorganisms and have been used in treating sores, gum disease, and wounds. Many researchers studied bactericidal activity using a different extract from *R. graveolens.* Moreover, *R. graveolens* have been reported previously that crude extract is capable to inhibit the growth of *Staphylococcus aureus, Salmonella typhimurium,* and *Bacillus subtilis* (Reddey and Al-Rajab 2016). However, secondary metabolites synthesized by the plants are responsible for their capability against microorganisms (Parray *et al*. 2012). Therefore, the impact of extract from different plants was studied by many researchers. Since many secondary metabolites and their cause are sources of antibacterial agents (Reddey and Al-Rajab 2016). Debnath (2008) indicated that plant crude extracts were found to be the initial steps for the screening of pure compounds that were isolated from exceptional outcomes. In addition, secondary metabolites extracted from tissue cultures may be more easily purified because of simple extraction procedures and the absence of significant amounts of interfering pigments, which will minimize the cost of purifying and producing such valuable compounds (Varma 2011). To our knowledge, the present study is the first report to illustrate an extensive study on *in vitro* propagation and antimicrobial activity of *R. graveolens.* Therefore, this study was conducted to develop a simple procedure for micropropagation, also to evaluate the antimicrobial activity of these important medicinal plants using the different solvent procedures.

Materials and Methods

Plant materials and culture conditions

R. graveolens plants seed were collected from five years plant from the Al-Sareeh, Irbid, Jordan (about 600 meters above sea level, 32.3306° N latitude and 35.8951°E Longitude). Then seeds were sterilized by using 4% NaOCl for 10 min, then implanted in 70% ethanol with shaking for 1 minute. After sterilization, seeds were then washed three times in deionized sterile water in a laminar flow cabinet. The seeds were germinated firstly on agar water media, then shoots were cultivated on Murashige and Skoog (MS) medium (1962). Agar was added at 8 g/L agar was prior to autoclaving. The medium pH was adjusted to 5.8. 80 mL of medium was dispensed in each 250 mL flask. Microshoots were incubated in the growth chamber at 24 ± 2ºC with a 16 h photoperiod and photosynthetic photon flux density (PPFD) of 50 μmol m^{-2} s^{-1} supplied by cool white fluorescent lamps.

Elongation of microshoot

Microshoots were subculture onto80 mL MS medium (250 mL flask) enriched with 0.05 mg/L 6-benzylaminopurine (BAP) and 30 g/L sucrose.

Effect of thidiazuron (TDZ) or zeatin on shoot proliferation

Microshoots length of 10 mm in length was cultivated on MS medium to enrich the various concentrations of TDZ and zeatin. Sixteen treatments were used and each treatment consisted of four microshoots. The culture was incubated as described above. Six weeks later data were collected on the number of shoots, shoot length, and leaves number.

Antimicrobial activity

Tested plants: *R. graveolens* plant material (i*n vitro* and *ex vitro*) was obtained from the tissue culture laboratory of Al-Balqa Applied University.

Fungal and bacterial growth media

The fungal strain was cultured on (15 mL were poured to 9 cm Petri dishes) potato dextrose agar (PDA; Himedia, India), while nutrient agar medium (NA; Fluka, Germany) was used for bacterial strains, about 15 mL (4 mm in thickness) were poured to 9 cm sterile Petri dishes.

Plant extractions

Plants materials (*in vitro* and *ex vitro*) (20 g) were dried in the shade for 14 d, using in liquid nitrogen (LN), ground to a fine powder, and then using 100 mL (methanol or ethanol) were extracted by soaking plant material for seven days (Ndukwe *et al.* 2006). Then using a rotary evaporator, the solvents were eliminated (HeidolphVV2000, Germany) under reduced pressure at below 50ºC temperatures. According to methods, two volumes were taken from the extract (40 μL or 80 μL) were disposed of in dimethylsulphoxide (DMSO) in (250 μg/μL) concentration and then were evaluated opposite microbe's activity, the crude extracts were stored at -20ºC until used. Both (bactericide) (oxytetracycline) and fungicide (cyclohexamine), were used as positive control and DMSO (controls) was used as negative. For evaluated their activity to reduce bacterial and fungal growth, methanol or ethanol extracts were dissolved in DMSO.

Antibacterial activity assay by the agar well diffusion method

Using sterile swap different bacterial strains were spread on

nutrient agar plates. A well divided into 4 quadrates on 6 mm diameter sterile plates with a sterile cork borer composed of different *R. graveolens* extracts at 40 μL or 80 μL at (250 μg/μL) concentration for 1.0 h. Then Petri dishes were cultivated at 37 ± 2°C for 24 h. Oxytetracycline (antibiotic) was used as a positive control, which was prepared in (250 μg/μL) and their antimicrobial activity was examined. The solvent dimethyl sulfoxide DMSO (negative control) was added, at the end of the cultivation growth period, the inhibition zone was scaled in mm. The diameter of the inhibition zone was determined by measuring the microbial compared with a standard antibiotic (Oxytetracycline). Three Petri dishes for each treatment were used which consisted of three replicates.

Antifungal activity assay by the agar well diffusion method

A 100 μL spore suspension (1×10^8 spores/mL) of an aliquot of each isolate was grooved on the surface in radial patterns on media plates. Each well was made on the plates divided into 4 quadrates (6 mm diameter), with a sterile cork borer which gains the different *R. graveolens* extracts. At 250 μg/μL, 40 and 80 μL *of R. graveolens* aliquots extract were enriched into wells and left for 1 h to diffuse, then the plates were incubated at 30 ± 2°C for 48 h. Positive control (Cyclohexamine) of the antifungal was prepared in 250 μg/μL and using the same manner the antimicrobial activity was tested. At the end of the cultivation growth period, the inhibition zone was scaled in mm. The solvent dimethyl sulfoxide DMSO (negative control) was added Three Petri dishes for each treatment were used which consisted of three replicates.

Statistical analysis

A completely randomized design was used in this study. The results data were exposed to ANOVA test. Duncan Multiple Range tests were used for mean separation. Data were analyzed using SPSS programs version 16 (SPSS 2007).

Results

Impact of thidiazuron (TDZ) and kinetine

TDZ at a concentration of 1.5 mg/L resulted in significantly the maximum number of shoots (9.44 shoots per explants) (Fig. 1). Shoot length increased significantly with increase TDZ concentration. The highest shoot length (23.06 mm) was produced at 2.0 mg/L. TDZ at 2.0 mg/L produced the maximum number of leaves (6.44 leaves per explants) (Table 1).

Impact of zeatin

3.75 shoots per explants ~~of~~ were gained when MS medium containing 2.0 mg/L zeatin was used (Table 2). While, on MS medium containing 1.0 mg/L zeatin, the maximum shoot length of 49.56 mm was promoted a maximum number of leaves per explant formed in medium containing 1.0 mg/L zeatin, the largest callus (more than 10 mm in diameter) was recorded on 1.5 mg/L zeatin about.

Table 1: Influence of thidiazuron (TDZ) on shoot number, shoot length, and leaves number of *in vitro R. graveolens* microshoots after six weeks growth periods

TDZ (mg/L)	Number new shoots/explant	Length of shoot (mm)	Leaves number /explant
0.0	1.13 ± 0.09 a	22.19 ± 1.76 c	4.06 ± 0.51 a
0.5	5.38 ± 0.93 b	10.00 ± 0.00 a	3.38 ± 0.22 a
1.0	6.25 ± 0.56 b	12.50 ± 1.71 ab	4.63 ± 0.40 a
1.5	9.44 ± 0.87 c	16.06 ± 1.46 b	6.38 ± 0.34 b
2.0	6.81 ± 0.96 b	23.06 ± 2.53 c	6.44 ± 0.71 b

Means followed by the same letter within the column are not significantly different according to Duncan Multiple range test at $P \leq 0.05$. Each treatment consisted of 16 replicates and each sample contained four microshoots**.** Values are the means ± standard error

Table 2: Influence of zeatin on shoot number, shoot length, and leaves number of *in vitro R. graveolens* microshoots after six weeks growth periods

Zeatin (mg/L)	Number new shoots/explant	Length of shoot (mm)	Leaves number /explant
0.0	1.13 ± 0.09 a	22.19 ± 1.76 a	4.06 ± 0.51 a
0.5	2.19 ± 0.26 b	40.75 ± 4.53 bc	10.00 ± 1.52 a
1.0	3.69 ± 0.52 c	49.56 ± 5.98 c	21.13 ± 5.64 b
1.5	3.56 ± 0.56 c	44.13 ± 3.52 c	10.19 ± 1.03 a
2.0	3.75 ± 0.62 c	31.75 ± 3.29 ab	9.56 ± 1.25 a

Means followed by the same letter within the column are not significantly different according to Duncan Multiple range test at $P \leq 0.05$. Each treatment consisted of 16 replicates and each sample contained four microshoots**.** Values are the means ± standard error

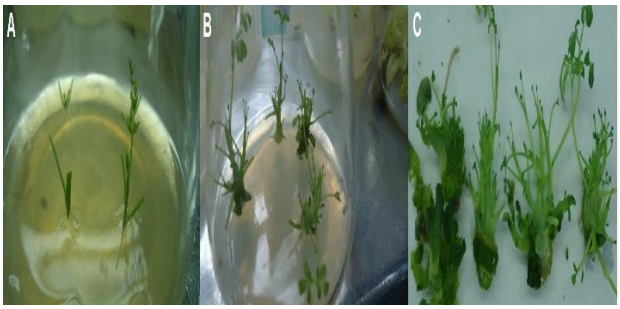

Fig. 1: Formation of multiple shoots of *R. graveolens* after different growth periods grew on MS media containing 1.5 mg/L TDZ. **A**) One-week growth period, **B**) 4 weeks' growth periods, **C**) 6 weeks' growth periods. Bars represent 5 mm

Antifungal activity

R. graveolens crude in *vitro* and *ex vitro* extract of invariant solvent shown to be very efficacious against bacteria and fungi. The methanolic and ethanolic extracts were effective against bacteria and fungi. However, the activities against some microbes were quite similar to a positive control (Tables 3–6). The *in vitro* ethanolic leaf crude extract was

Table 3: Impact of various plant types of *Ruta graveolens* using ethanolic extract against different fungal species

Fungal strain	Crude amount (μL)	Zone of inhibition (mm)			
		Ethanol		Control	
		Ex vitro	*In vitro*	Positive	Negative
P. digitatum	40	26.33 ± 1.45 cd	21.67 ± 2.03 d	17.33 ± 1.45 cde	0.00 ± 0.00 a
	80	31.00 ± 1.73 de	28.00 ± 1.73 e	22.00 ± 1.73ef	0.00 ± 0.00 a
A. niger mutant. brown	40	14.00 ± 1.53 b	17.67 ± 1.76 c	9.33 ± 1.45 a	0.00 ± 0.00 a
	80	21.33 ± 2.03 c	32.67 ± 2.60 fg	20.33 ± 1.45 e	0.00 ± 0.00 a
A. niger mutant. black	40	0.00 ± 0.00 a	0.00 ± 0.00 a	25.33 ± 1.45 f	0.00 ± 0.00 a
	80	15.33 ± 2.03 b	12.00 ± 1.15 b	32.00 ± 1.73 g	0.00 ± 0.00 a
P. chrysogenum	40	11.33 ± 2.03 b	0.00 ± 0.00 a	11.00 ± 1.73 ab	0.00 ± 0.00 a
	80	21.00 ± 1.73 c	0.00 ± 0.00 a	15.33 ± 1.45 bcd	0.00 ± 0.00 a
P. italicum	40	21.00 ± 2.31 c	0.00 ± 0.00 a	10.00 ± 1.73 a	0.00 ± 0.00 a
	80	27.00 ± 1.73 d	0.00 ± 0.00 a	13.67 ± 1.20 abc	0.00 ± 0.00 a
A. nidulans	40	10.67 ± 1.76 b	0.00 ± 0.00 a	10.33 ± 0.88 a	0.00 ± 0.00 a
	80	14.00 ± 1.53 b	0.00 ± 0.00 a	15.33 ± 1.45 bcd	0.00 ± 0.00 a
C. albicans	40	26.67 ± 2.03 d	30.00 ± 1.53 ef	11.33 ± 1.45 ab	0.00 ± 0.00 a
	80	34.33 ± 1.45 e	36.00 ± 1.73 g	19.33 ± 1.45 de	0.00 ± 0.00 a

Means followed by the same letter within the column are not significantly different according to Duncan Multiple 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 48 hours on PDA media

Table 4: Impact of various plant types of *Ruta graveolens* using methanolic extract against different fungal species

Fungal strain	Crude amount (μL)	Zone of inhibition (mm)			
		Methanol		Control	
		Ex vitro	*In vitro*	Positive	Negative
P. digitatum	40	23.00 ± 1.15 d	26.67 ± 2.03 def	17.33 ± 1.45 cde	0.00 ± 0.00 a
	80	28.00 ± 1.73 e	33.33 ± 2.03 g	22.00 ± 1.73ef	0.00 ± 0.00 a
A. niger mutant. Brown	40	11.67 ± 1.76 ab	25.00 ± 2.31 de	9.33 ± 1.45 a	0.00 ± 0.00 a
	80	15.00 ± 1.73 b	29.67 ± 1.76 efg	20.33 ± 1.45 e	0.00 ± 0.00 a
A. niger mutant. Black	40	16.33 ± 1.45 bc	11.67 ± 1.76 bc	25.33 ± 1.45 f	0.00 ± 0.00 a
	80	22.00 ± 1.73 d	16.00 ± 1.73 c	32.00 ± 1.73 g	0.00 ± 0.00 a
P. chrysogenum	40	13.00 ± 1.73 ab	0.00 ± 0.00 a	11.00 ± 1.73 ab	0.00 ± 0.00 a
	80	20.33 ± 1.45 cd	0.00 ± 0.00 a	15.33 ± 1.45 bcd	0.00 ± 0.00 a
P. italicum	40	12.33 ± 1.45 ab	23.33 ± 2.03 d	10.00 ± 1.73 a	0.00 ± 0.00 a
	80	20.33 ± 2.03 cd	29.33 ± 2.03 efg	13.67 ± 1.20 abc	0.00 ± 0.00 a
A. nidulans	40	8.33 ± 0.88 a	7.33 ± 0.88 b	10.33 ± 0.88 a	0.00 ± 0.00 a
	80	12.00 ± 1.73 ab	13.33 ± 1.45 c	15.33 ± 1.45 bcd	0.00 ± 0.00 a
C. albicans	40	29.00 ± 2.31 e	31.00 ± 1.73 fg	11.33 ± 1.45 ab	0.00 ± 0.00 a
	80	36.33 ± 2.03 f	39.00 ± 1.73 h	19.33 ± 1.45 de	0.00 ± 0.00 a

Means followed by the same letter within the column are not significantly different according to Duncan Multiple 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 48 hours on PDA media

not identifying to be effective for some fungi including *Penicillium chrysogenum*, *P.* italicum, and *Aspergillus nidulans*, On the other hand, methanolic extract was not effective against *P. chrysogenum*. Maximum inhibition with ethanolic extract was found against *Candida albicans*, *P. digitatum* and *P. italicum*. While methanolic leaf crude extract was found to be active against *C. albicans*, *P. digitatum*, and *P. italicum*. The activity of the ethanolic and methanolic extract was similar to the antifungal agent tested (Tables 3 and 4). Both *ex vitro* and *in vitro* extract showed to be similar in antifungal properties.

Antibacterial activity

Crude *ex vitro* and *in vitro* extract was very effective against some bacterial species. *In vitro* leaf extract showed a higher inhibition zone against the *A. niger mutant. brown* compared with *ex vitro* plants. The antibacterial activities of methanolic extract and ethanolic leaf crude extracts compared with an antibiotic used were represented in (Tables 5 and 6). The results of the antibacterial activity showed that *ex vitro* and *in vitro* extracts were more efficacious apposite to Gram-positive and Gram-negative bacteria. On the contrary, Gram-negative bacteria were more resistant. *In vitro* and *ex vitro* methanolic and ethanolic crude leaf extracts showed similar results (Tables 5 and 6).

R. graveolens in vitro and *ex vitro* leaf crude ethanolic extract was more effective against Gram-positive bacteria tested. Ethanolic and methanolic *in vitro* leaf extracts showed a varying degree of inhibition against bacteria. Maximum inhibition was found in *S. aureus*, followed by *Micrococcus latus* and then *B. cereus*. The results of the current study show that the solvent used plays an important role in the antimicrobial efficacious. Moreover, the *ex vitro* and *in vitro* leaf extracts showed similar antibacterial properties.

Discussion

In vitro R. graveolens plantlets were established successfully, with only a very low contamination percentage (data not shown). The sterilization procedure used in this

Table 5: Impact of various plant types of *Ruta graveolens* using methanolic extract against different fungal species

Strain	Crude amount (µL)	Zone of inhibition (mm)			
		methanol		Control	
		Ex vitro	*In vitro*	Positive	Negative
S. aureus	40	20.33 ± 1.45 d	30.33 ± 1.45 d	34.67 ± 0.88 d	0.00 ± 0.00 a
	80	22.67 ± 1.45 d	33.00 ± 2.08 d	41.00 ± 1.15 e	0.00 ± 0.00 a
B. cereus	40	20.67 ± 1.20 d	25.67 ± 1.20 c	30.67 ± 1.20 c	0.00 ± 0.00 a
	80	22.00 ± 1.73 d	30.67 ± 1.20 d	36.33 ± 1.20 d	0.00 ± 0.00 a
Micrococcus latus	40	15.33 ± 1.20 c	30.33 ± 1.45 d	35.67 ± 0.88 d	0.00 ± 0.00 a
	80	22.33 ± 1.76 d	33.33 ± 1.03 d	43.67 ± 1.20 e	0.00 ± 0.00 a
Salmonella typhimurium	40	0.00 ± 0.00 a	0.00 ± 0.00 a	10.67 ± 1.20 a	0.00 ± 0.00 a
	80	0.00 ± 0.00 a	0.00 ± 0.00 a	12.67 ± 1.20 a	0.00 ± 0.00 a
Pseudomonas aeruginosa	40	0.00 ± 0.00 a	0.00 ± 0.00 a	13.33 ± 1.20 a	0.00 ± 0.00 a
	80	0.00 ± 0.00 a	0.00 ± 0.00 a	24.00 ± 1.53 b	0.00 ± 0.00 a
Escherichia coli	40	11.67 ± 1.76 b	12.00 ± 2.08 b	24.33 ± 1.20 b	0.00 ± 0.00 a
	80	15.33 ± 0.88 c	1 4.00 ± 1.15 b	50.33 ± 0.88 f	0.00 ± 0.00 a

Means followed by the same letter within the column are not significantly different according to Duncan Multiple 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 24 hours on NA media

Table 6: Impact of various plant types of *Ruta graveolens* using ethanolic extract against different bacterial species

Strain	Crude amount (µL)	Zone of inhibition (mm)			
		Ethanol		Control	
		Ex vitro	*In vitro*	Positive	Negative
S. aureus	40	19.33 ± 2.03 c	30.33 ± 1.76 c	34.67 ± 0.88 d	0.00 ± 0.00 a
	80	21.67 ± 1.76 c	34.00 ± 1.53 d	41.00 ± 1.15 e	0.00 ± 0.00 a
B. cereus	40	20.33 ± 0.88 c	28.33 ± 1.45 c	30.67 ± 1.20 c	0.00 ± 0.00 a
	80	20.33 ± 1.45 c	30.00 ± 1.73 c	36.33 ± 1.20 d	0.00 ± 0.00 a
Micrococcus latus	40	28.33 ± 1.45 d	12.00 ± 1.73 b	35.67 ± 0.88 d	0.00 ± 0.00 a
	80	34.67 ± 1.20 e	14.33 ± 1.45 b	43.67 ± 1.20 e	0.00 ± 0.00 a
S. typhimurium	40	0.00 ± 0.00 a	0.00 ± 0.00 a	10.67 ± 1.20 a	0.00 ± 0.00 a
	80	0.00 ± 0.00 a	0.00 ± 0.00 a	12.67 ± 1.20 a	0.00 ± 0.00 a
P. aeruginosa	40	0.00 ± 0.00 a	0.00 ± 0.00 a	13.33 ± 1.20 a	0.00 ± 0.00 a
	80	0.00 ± 0.00 a	0.00 ± 0.00 a	24.00 ± 1.53 b	0.00 ± 0.00 a
E. coli	40	0.00 ± 0.00 a	0.00 ± 0.00 a	24.33 ± 1.20 b	0.00 ± 0.00 a
	80	9.00 ± 1.15 b	12.67 ± 1.20 b	50.33 ± 0.88 f	0.00 ± 0.00 a

Means followed by the same letter within the column are not significantly different according to Duncan Multiple 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 24 hours on NA media

study gave satisfactory results. Micropropagation has been used to promote conservation and maintenance of free disease-free plants under controlled environmental conditions (Luan *et al*. 2006; Alrayes *et al*. 2018). Huettema and Precee (1993) reported that low concentrations (<1 μM) increased axillary proliferation compared to other cytokines. on the other hand, TDZ inhibits the length of the shoot. Moreover, TDZ can enhance adventitious shoots, somatic embryos and the formation of callus. The adding of different concentrations of TDZ or zeatin to the media of *R. graveolens* enhance multiplication (Tables 1 and 2). In the current study TDZ promotes multiple shoots (Table 1).

Medium containing 1.5 mg/L TDZ produced maximum number of shoots (9.4 shoots per explants). However, this study showed that TDZ at low concentrations had efficiency in the production of adventitious buds (Murthy *et al.* 1998). This is opposite to the previous finding in *Artemisea herba-alb and Stevia rebaudiana* Shatnawi *et al*. 2011a, b. On the other hand, TDZ increased the axillary bud formation and decreased the length of the newly developed shoot because it released apical dominance. Reddy *et al*. (2015) reported that shoot sprouting percentage, shoot number, and length were increased with increasing TDZ concentration. Therefore, the TDZ effect depends on exposure duration, explant, and plant (Huettema and Preece 1993; Reddy *et al*. 2015).

Antimicrobial activity

Nowadays there is a high demand for discovering new substances from plants to use against many microorganisms, which can affect human health. *R. graveolens ex vitro* leaf showed effectiveness against both bacteria and fungi. *R. graveolens* i*n vitro* and *ex vitro* leaf extracts of *R. graveolens* prepared in ethanol and methanol showed high activity against microorganisms tested (Tables 3-6). However, both ethanol and methanol extracts were found to be positive against gram-positive bacteria and gram-negative bacteria. The methanolic extract showed the high effective opposite to the bacteria study (*S. aureus, B. cereus, Micrococcus latus,* and *S. typhimurium* the activity was quite similar to antibiotic tested. Using *in vitro* ethanolic plants extract maximum inhibition was found in *Micrococcus latus*, followed *B. cereus*, and *S. aureus* (Table 6). While using *in vitro* ethanol plant extract with fungi, the highest zone of inhibition was found in *C. albicans* followed

P. digitatum, P. italicum and A. niger mutant. brown. The activity of the *in vitro* extract using methanol with fungi was found to be *C. albicans*, *P. digitatum*, *A. niger mutant. black*, and *P. italicum.*

In this study, the methanolic extract of *R. gra*veolens had an antimicrobial effect in accordance with the other investigations realized on different kinds of explants (Ojala *et al.* 2000*;* Oliva *et al.* 2003). The methanolic and ethanolic extract shows effectiveness against fungi tested in this study where the activity was similar to then antifungal tested. *R. graveolens* leaves would be useful in developing antimicrobial substances. *R. graveolens* extract might have the mode of action on DNA strands that cause cell death. Preethi *et al.* (2008) indicated that *R. graveolens* at higher concentrations acted as a pro-oxidant rather than an antioxidant, which influences mitochondrial absorbency transition pore (Kushnareva and Sokolove 2000; Preethi *et al*. 2006). This plant has strong antispasmodic properties. *R. graveolens* plants accumulate linear furanocoumarins (psoralens) and acridone or furoquinolone alkaloids. The acridone alkaloids were detected in all organs particularly in endodermal and vascular tissue (Kushnareva and Sokolove 2000; Preethi e*t al*. 2006).

In vitro extracts show high antimicrobial activity against tested bacterial species; it may be due to the presence of a high concentration of toxic compounds as result in HPLC analysis (Al-Ajlouni *et al.* 2015). It has been reported it may contain flavonoids rutin, alkaloids quinolone, furoquinolone, acridone, (psoralens), essential oils like 2-nananone, 2- undecyl acetate, graveoline, coumarins like furocoumarin pyranocoumarin and (Sinshemoke *et al*. 2000; Preethi *et al*. 2006). This may be attributed to the cause of its high antimicrobial activity because it contains different secondary metabolites. In conclusion, this study builds up good evidence that *R. graveolens* may possibly be used as natural medical utilization for microbes and would help for the development of a new alternative medicine system that has no side effects.

Conclusion

The present study has resulted in the founding of a consistent and reproducible protocol of *R. graveolens* which could be used for mass multiplication as well as antibacterial and antifungal activity against both some bacteria and fungi. *In vitro, R. graveolens* plantlets were established successfully. Moreover, TDZ at 1.5 mg/L resulted in significantly the maximum number of shoots (9.44 shoots per explants). The crude leaf extract from *in vitro* and *ex vitro R. graveolens* dissolved in different solvent solutions showed diverse activity against both some bacteria and fungi. Moreover, this study builds up worthy indication that *R. graveolens* may possibly be used as normal medical utilization for microbes and would help for the expansion of a new alternative medicine system that has no side effects.

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Author Contributions

MA, MS, and WS planned the experiments, and sample collection. SA, MM and SAb interpreted the results, MA, MS, MM and WS made the write original, editing, and statistically analyzed the data, and made illustrations. All authors commented on the manuscript, reviewed drafts of the paper, and approved the final draft.

Conflict of Interest

All authors declare no conflict of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Ethics Approval

Not applicable in this manuscript

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