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Investigation the Antibacterial Activity of *Portulaca oleracea* L. Tissue Cultures *in vitro*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AGO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAA and RAM managed the analyses of the study. Author WJO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this research was to studying the antibacterial activity of *Portulaca oleracea* L. *in vitro* cultures. *P. oleracea* leaves were collected from the local markets. Callus tissues were induced from the plant leaves (expalnt) through inoculating the explants on MS medium supplemented with different concentrations of 2, 4-D (0.0, 2.0 or 3.0 mg/l) and Kintin (0.0 or 0.5 mg/l). All cultures were incubated at 25 ± 2°C in dark. Water and ethanol extracts of *P. oleracea* were prepared. Bacterial samples were collected, they consist of gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Pseudomonas aeruginosa*). Antibacterail activity of *P. oleracea* extracts were investigated using diffusion method. Results shown that mean % callus induction, callus fresh weight and callus dry weight inceasred significantly in 2.0 mg/l 2, 4-D or 3.0 mg/l 2, 4-D with 0.5 mg/l kin in comparsion to the control (0.0), and the highest mean obtained at 3.0 mg/l 2, 4-D with 0.5 mg/l kin recording 80% and 94 mg and 52 mg for % callus induction, callus fresh weight and callus dry weight respectively. Also there was no significat

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differences ocurred in the mean inhibition zone diameter (mm) of the bacterial isolates treated with water or ethanol extracts of callus tissues recording 10 and 9.5 mm respectively compard with control (14.5 mm) and mean inhibition zone (mm) significantly decreased in other treatment types in compasion to the control. The results obtained in this research work clearly indicated a promising potential of *P. oleracea* extracts as antibacterial agent and inspite of the small inhibition zone diameter obtaind using plant extract but it has proved highly efficiency as an anti bacterial compared with the higher concentration of the standard antibiotic used in this experiment.

Keywords: Portulaca oleracea; plant tissue culture; antibacteria activity.

1. INTRODUCTION

Portulaca oleracea L. (Purslane) is a medicinal plant found in Europe and Asia. It contains plenty of bioconstituents, including catecholamines, Inoradrenalin and dopamine and using in treatment of urinary, digestive problems, and cardiovascular diseases. *P. oleracea* has a variety of pharmacological activities, including analgesic, anti-inflammatory, antimicrobial, and wound healing and hypoglycemic [1,2,3]. Information about tissue culture of this plant is comparatively less. Safdari and Kazemitabar [4] reported some tissue culture work of this plant and they studied on two races of purslane; agronomic purslane and wild one, and they concluded that there are tangible differences between these two types of purslane in response to tissue culture approaches, such that different explants or hormonal treatments were suitable to different aims for each type. Medicinal plants and herbs have long been used in treating diseases and illnesses [5]. *P. oleracea* L. has achieved almost identical recognition in each culture for its benefits. The use of this plant as a vegetable, spice and medicinal plant has been known since the times of the ancient Egyptians and was popular in England during the middle Ages [6]. Its contains more omega-3 fatty acids (alpha-linolenic acid in particular) than any other leafy vegetable plant. Studies have found that purslane has 0.01 mg/g of eicosapentaenoic acid (EPA). It also contains vitamins (mainly vitamin A, vitamin C, vitamin E (alpha-tocopherol), vitamin B, carotenoids), and dietary minerals such as magnesium, calcium, potassium, and iron. Also present of two types of betalain alkaloid pigments, the reddish betacyanins (visible in the coloration of the stems) and the yellow betaxanthins (noticeable in the flowers and in the slight yellowish cast of the leaves). Both of these pigment types are potent antioxidants and have been found to have antimutagenic properties [7], Betacyanins isolated from *Portulaca oleracea* improved cognition deficits in aged mice. A subclass of homoisoflavonoids from the plant showed *in vitro* cytotoxic activities towards four human cancer cell lines [8]. *P. oleracea* is a commonly found species and a medicinal food for human consumption [9]. The stems and leaves of the plant are succulent and edible with a slightly acidic and salty taste similar to spinach. It is a reasonable choice due to its high nutritive and antioxidant properties as human food, animal feed and medical utilization and it is believed that the regular consumption of dietary antioxidants may reduce the risk of several serious diseases [10]. It was found that the plant has many bioactive compounds which include flavonoids, Alkaloids, Saponin, Tanin and Cardiac glycoside. The presence of these phytochemical groups in most plants has been reported to have medicinal properties. It's also used for treatment of burns, headache, liver, stomach, cough, shortness of breath, diseases related to the intestine and arthritis [11].

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

P. oleracea leaves were collected from the local markets in Baghdad city in October 2016.

2.2 Medium Preparation for Callus Induction

Murashige and Skoog (MS) medium were prepared and supplemented with sucrose (3%), myo-inositol and growth regulators. The pH of the medium was adjusted to 5.8 using 0.1N NaOH or 0.1N HCl, then 8 g/l agar (Sigma-Aldrich) was added to the medium. The medium was dispensed into 15x2.5 cm tubes (vials) (10 ml/tube).

2.3 Medium Sterilization

The medium was sterilized by autoclaving (Viseclave-MACS-1100, Korea) at 15 lbs pressure and 121°C for 15 min.

2.4 Induction of *P. Oleracea* Callus Cultures

P. oleracea leaves were surface-sterilized in 50% (v/v) commercial bleach for 3 min and subsequently rinsed in distilled sterile water (3×3 min). Then they were inoculated on MS medium supplemented with different concentrations of 2, 4-Dichlorophenoxyacetic acid (2, 4-D) (0.0, 2.0 or 3.0 mg/l) and Kintin (kin) (0.0, 0.5 mg/l). All cultures were incubated at 25 ± 2°C in dark [12]. Leaves were cultured by placing 1-3 explant using 10 replicates for each treatment. After four weeks of incubation, callus induction frequency (%) was calculated using the following formula:

Callus induction frequency (%) = No of explant produced callus/total explants cultured x100 [13].

2.5 Preparation of Extracts

Leaves and callus tissues of *P. Oleracea* were dehydrated by air-drying at ambient temperature of 23-25°C in the dark in order to avoid the degradation of pigments and polyphenolic compounds. The dried tissues were grinding well then 50 mg of the powder was soaked in 100 ml of sterilized distilled water or 100 ml of 70% ethanol using magnetic stirrer for 24 hrs at room temperature. Extracts were filtered through Whatman filter paper No.1 then it was concentrated in vacuum at 40°C using a rotary evaporator. The dried extracts were stored properly [12].

2.6 Sterilization of Extracts

P. oleracea extracts were sterilized using sterile disposable Millipore filter units (0.20 µm in diameter) in a laminar air flow cabinet.

2.7 Measurement of Bacterial Cultures Concentration

Bacterial isolates were supplemented from bacterial isolates bank in Microbiology Lab., Post graduate laboratories, College of Applied Science, Al-Nahrain university, at which single colonies from cultures grown on nutrient agar for 18-24 hrs were transferred to test tubes containing 5 ml of normal saline and mixed well by vortex, then bacterial growth was compared with McFarland tube No.0.5 turbidity standard solution, which was equivalent to a bacterial inoculum concentration of 1.5 X $10^{\wedge 8}$ cell / ml.

2.8 Determination of Inhibition Zones of Treatments

By using cotton swab, a touch of bacterial culture (broth) was transferred to Muller Hinton agar medium and streaked three times by rotating the plate approximately 60º between streaking. To ensure even distribution of the inoculum. The inoculated plates were placed at room temperature for 10 min to allow absorption of excess moisture [14]. Then, using sterilized pauster pipette for making wells (the wells were arranged so as to avoid the development of overlapping of inhibition zones) which were then filled with 25 µl of the sterilized plant extracts. The plates were incubated at 37°C for 18-24 hrs. After incubation, inhibition zones were measured using ruler for determination of their diameters in millimeters. Standard antibiotic used was streptomycine disc and the plates were also incubated at 37°C for 18-24 hrs.

2.9 Experimental Design and Statistical Analysis

The experiments were designed as factorial experiments with a completely randomized design**.** Analyses were done using the SPSS var. 12 software. Differences between means were determined and least significant differences were compared at $P \leq 0.05$ [15].

3. RESULTS AND DISCUSSION

3.1 Effect of Different Concentrations of Plant Growth Regulators on % Callus Induction, Callus Fresh Weight and Callus Dry Weight of *Portulaca oleracea* Plant

Data in Tables 1, 2 and 3 exhibited that mean % callus induction, callus fresh weight and callus dry weight inceasred significantly in 2.0 mg/l 2,4-D and 0.5 mg/l kin, 3.0 mg/l 2,4-D or 3.0 mg/l 2,4-D and 0.5 mg/l kin in comparsion to the control (0.0), and the highest mean obtained at 3.0 mg/l 2,4-D and 0.5 mg/l kin recording 80%, 94 mg and 52 mg for % callus induction, callus fresh weight and callus dry weight respectively and these results were in agreement with those obtained by Zhenxia et al. [16] who exhibited that the optimum media for callus induction from leaves of *P. oleracea* are MS+2.0 mg/L 6- Benzyl adenine purin (BAP) and MS+2.0 mg/L 6- BAP + 1.0 mg/L Naphthaleneacetic acid (NAA) at 30±2℃ respectively. Safdari and Kazemitabar [4]

reported that the treatments containing10 µM Indole 3-butyric acid (IBA) in combination with 10 or 5 µM BAP are suitable for callus induction from leaves of wild purslane. Direct shoot regeneration from shoot tips or petiole explants of wild purslane was observed only in 10 µM IBA alone. Also, BAP at level 8.88 µM was found to be the best treatment to shoot regeneration from nodal segments of agronomic purslane and IBA at level 2.5 µM was found to be the best treatment for rooting of regenerated shoots in both races of purslane. While Safdari and Kazemitabar [17] investigated that combination with 10 or 5 µM BAP were found to be suitable treatments for callus production from leaf explants, as well. Moreover, 15 µM BAP alone or in combination with 5 µM NAA were found to be the best treatments for shoot regeneration from callus. The treatments containing different ratios of BAP and 2, 4-D hormones, caused callus formation in varying degrees. The medium containing 1 mg/L BAP and 1 mg/L of 2, 4-D hormones showed the higher production of callus [18]. Fig. 1 shown callus tissues that induced throught inoculating the explant on to MS media suplimented with different concentrations of growth regulators.

Table 1. Effect of different concentrations of plant growth regulators on mean % callus induction after inoculating explants for six weeks. n=10

Kin (mg/l) \ 2,4-D (mg/l)	0.0	2.0	3.0	Mean
0.0	0	0	30	10
0.5	0	60	80	46.6
Mean	0	30	55	
L.S.D 0.05		24.16		

Table 2. Effect of different concentrations of plant growth regulators on mean callus fresh weight (mg) after inoculating explants for six weeks. n=10

Kin (mg/l) \ 2,4-D (mg/l)	0.0	2.0	3.0	Mean
0.0	0	0	38	12.6
0.5	0	59	94	51
Mean	0	29.5	66	
L.S.D 0.05		19.263		

Table 3. Effect of different concentrations of plant growth regulators on mean callus dry weight (mg), after inoculating explants for six weeks. n=10

Kin (mg/l) \ 2,4-D (mg/l)	0.0	2.0	3.0	Mean
0.0	0	0	11	3.6
0.5	0	28	52	26.6
Mean	0	14	31.5	
L.S.D 0.05		20.166		

3.2 Antibacterial Activity of Water and Ethanol Extracts of Intact Plant and Callus Cultures

Results in Table 4 shown that there was no significat differences ocurred in the mean inhibition zone diameter (mm) of the bacterial isolates treated with water or ethanol extracts of callus tissues recording 10 and 9.5 mm zone of inhibition diameter respectively compard with control (14.5 mm). Also there was a significant decrease observed in the mean inhibition zone (mm) in all treatment types in compasion to the control and these results was on the line of those obtained by Londonkar and Nayaka [19] who studied the antimicrobial activity of *P. oleracea* leaves chloroform and ethanolic extracts by agar diffusion method against different types of microorganisms (*Staphyllococcus aureus, Bacillus cereus, Klebisilla pneumonia*, *Aspergillus fumigates* and *Nerospora crassa*) and they found that ethanol crude extract showed maximum effect against these bacteria except *K. pneumonia* which revealed moderate effect. Pallavi and Siddheshwar [20] studied the inhibitory effects of *Acmella oleracea* L. leaves against eight different bacteria *viz. Bacillus megaterium, B. subtilis, B. cereus, Escherichia coli, Klebsiella pneumonaie, Micrococcus luteus, Nocardia* sp. and *Pseudomonas aeruginosa,* after 24 hrs incubation it is observed that the plant extracts have good inhibitory action against the bacteria except *Nocardia* sp. Also aqueous and ethanolic extracts of *P. oleracea* displayed the highest antibacterial activities against *Escherichia coli* than other bacteria. For, ethanolic extracts had higher antibacterial activities than aqueous extracts except against *Escherichia coli* [21]. While Ercisli et al. [22] mentioned that *P. oleracea* leaves methanol extracts showed antibacterial activities against *Bacillus subtilis*, *Pseudomonas syringae* pv. Tomato, *Vibrio*

cholerae and *Yersinia pseudotuberculosis*. None of the water extracts showed antibacterial activity against the microorganisms studied. Christian et al. [23] reported that the extracts of P*. oleracea* showed significant activity against *S. aureus, S. pyogenes, E. coli, P. aeruginosa, and C. albicans* with MIC of 12.5, 12.5, 50, 50 and 50 mg/mL. In another study Zhao et al. [24] suggested that *P. oleracea* extract supplementation significantly altered the cecal bacterial community without affecting the intestinal pH. Fig. 2 shown the inhibition zone of *Pseudomonas aeruginosa* and Staphylococcus *aureus* as a result of treatment with *P. oleracea* leaves or callus culture extracted with water and ethanol.

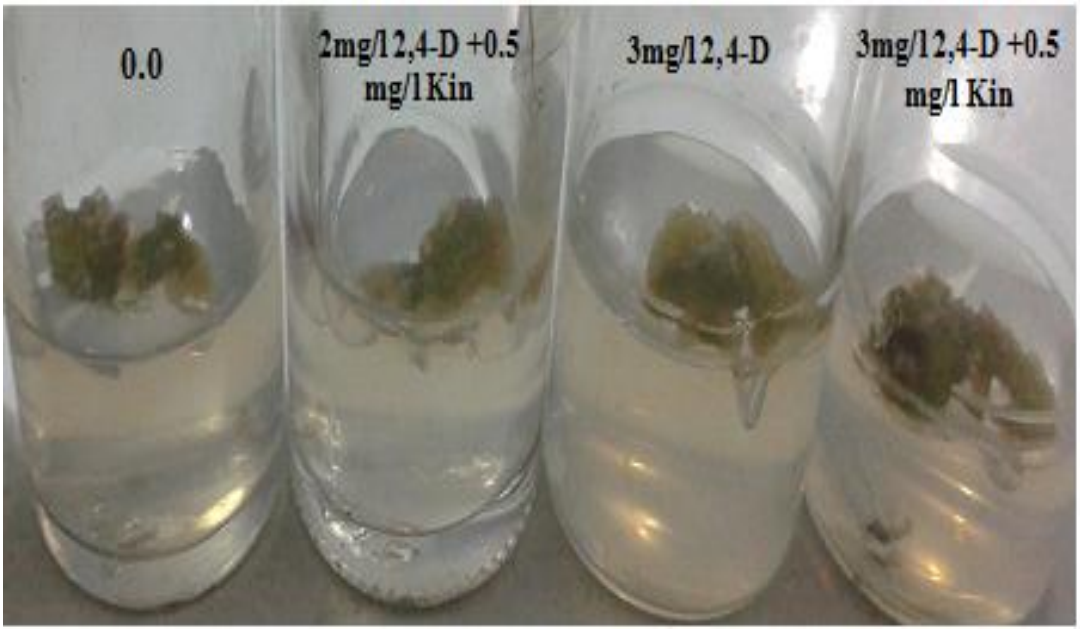

Fig. 1. *Portulaca oleracea* callus cultures originated from leaves that inoculated on to MS media suplimented with different concentrations of growth regulators, showing the changes in the callus mass after six weeks of inoculation, n=10

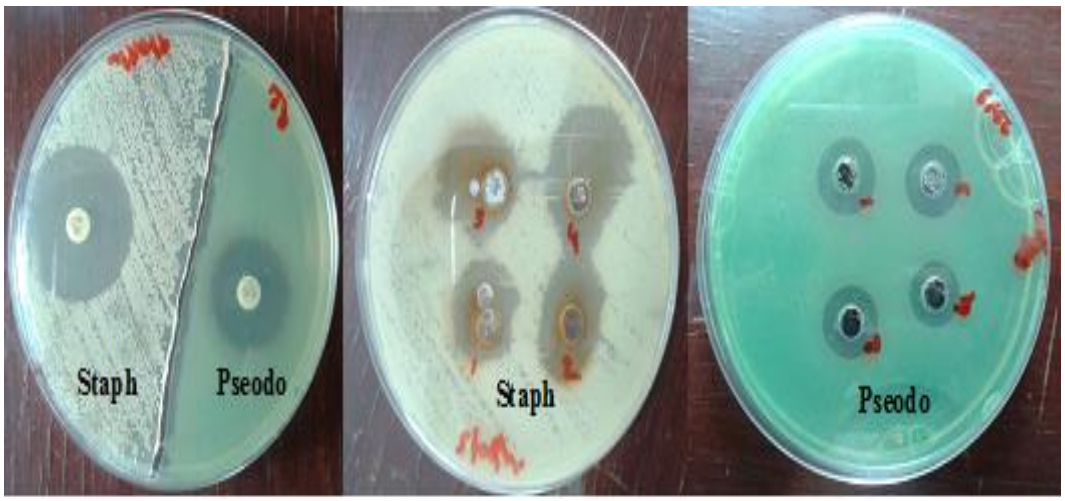

Fig. 2. Inhibition zone of *Pseudomonas aeruginosa* and *Staphylococcus aureus* as a result of treatment with P*. oleracea* leaves or callus tissues water and ethanol extracts. n=15

1 and 2= Water and ethanol extracts of P. oleracea leaves respactively
3 and 4= Water and ethanol extracts of P. oleracea callus tissues respactively

Table 4. Inhibition zones diameters (mm) of two types of bacteria treated with *Portulaca oleracea* leaves or callus tissues water and ethanol extracts. n=15

Type of bacteria / Type of treatment		*Pseudomonas aeruginosa*	*Staphylococcus aureus*	Mean
Control		19.0	10.0	14.5
Leaves	**Water extract**	11.0	5.0	8
	Ethanol extract	9.0	7.0	8
Callus tissues	**Water extract**	12.0	8.0	10
	Ethanol extract	11.0	8.0	9.5
L.S.D 0.05			6.242	

4. CONCLUSION

The results indicated that extracts of *P. oleracea* L. have the potential to be used as antibacterial agent and inspit of the small inhibition zone diameter obtaind using plant extract but it has proved highly efficiency as an anti bacterial compared with the higher concentration of the standard antibiotic used in this experiment. Further research is required to investigate the bioactive molecules of *Portulaca oleracea* L. and their clinical outcome as anti microbail agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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