

RESEARCH PAPER

Effects of *Prosopis farcta* (Banks de Sol.) Macbride Extracts Against Some Pathogenic bacteria and Seed Germination Indices of *Triticum aestivum*

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ABSTRACT:

This study was aimed to investigate the antimicrobial activities of aqueous and ethanolic extracts of *Prosopis farcta* fruits against some gram-positive and gram-negative bacteria in addition to the allelopathic effects on *Triticum aestivum* by investigating seed germination indices. Fruits of *P. farcta* were collected in Ballakayaty, Iraq's Kurdistan Region. Aqueous and ethanolic extracts were prepared at the concentrations 2048, 1024, 512, 256, and 128 µg/ml. The minimum inhibitory concentration (MIC) of the extracts were determined for each test bacteria. Well diffusion method was applied to determine the antibiotic susceptibility. The main results of this study demonstrated the antimicrobial activity of *P. farcta* extracts against some bacteria. For most used bacteria, the best concentrations were 512 and 256 µg/ml respectively, which had the lowest inhibitory concentration. When compared to controls (distilled water and pure ethanol 96%), the ethanolic extract of *P. farcta* at 512 µg/ml demonstrated the best seed germination index (39.167±4.902), while at 2048 µg/ml, the lowest seed germination index (11.667±4.944) was observed. However, the *P. farcta* aqueous extract at 256 µg/ml showed the highest seed germination index (60.667±1.801), and at 2048 µg/ml demonstrated the lowest seed germination index (30.833±1.537). According to the observed data the *P. farcta* aqueous extract showed the greatest level of root elongation. Root elongation was gradually reduced as concentrations increased because *P. farcta*'s ethanolic extract had a lower root elongation percentage. The ethanolic extract of *P. farcta* had a stronger depressing influence on the *T. aestivum* seedling vigor index.

KEY WORDS: *Prosopis farcta*; Antimicrobial activity; Pathogens; Germination indices; *Triticum aestivum*.

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1. INTRODUCTION

Infectious diseases are now problem in developing and developed countries which caused by various pathogenic microbes like bacteria, fungi, parasites and viruses, etc. Medicinal plants have been used against pathogenic microbes Herbal medicines are generally used for healthcare because they are wealthy source of antimicrobial properties and have low price (Khan *et al.*, 2019). Natural therapeutic agents of plant origin have been increasingly used to produce new and effective drugs, thus they have been commonly used as alternative medicine for the treatment and prevention of various diseases like cancer, diabetes and infectious diseases (García-Andrade *et al.*, 2013).

Members of the genus *Prosopis* are native to Africa, America and Asia, and have long been used in traditional medicine. Commonly, most species of *Prosopis* are used for medicinal purposes including *P. alba*, *P. africana*, *P. farcta*, *P. glandulosa*, *P. cineraria*, *P. juliflora*, *P. ruscifolia*, *P. nigra* and *P. spicigera* which are highly effective in birth/postpartum pains, asthma, otitis, conjunctivitis, diabetes, malaria, callouses, diarrhea, fever, flu, expectorant, lactation, liver infection, pains, rheumatism, scabies, skin inflammations, pediculosis, spasm, stomach ache, bladder and pancreas stone (Sharifi-Rad *et al.*, 2019).

Prosopis farcta (Banks de Sol.) Macbride is a useful and valuable medicinal plant for extracting flavonoids and it is one of the most important plants with medicinal properties. *P. farcta* is

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found in many southern regions of Iraq and Iran (Dheeb *et al.*, 2020).

To minimize human risk to infectious diseases that caused by pathogenic bacteria, the search for substances with strong antimicrobial activity has been one of the most intensive field of research (Mohamed *et al.*, 2020).

Germination considered as the initial stage of a plant's life cycle and determines where and when a crop can be established. It is a complex metabolic process that oxidizes the carbohydrates and lipids within the seed and breaks down storage proteins in order to obtain energy and amino acids necessary for plant development (Tsegay and Gebreslassie, 2014). Seed plays an important role in seed germination process because it is the reproductive unit and considered as the thread of life that assures the survival of all plant species as well as seed play role in standing establishment, thus seed germination remains a key to modern agriculture. Thus, especially in a world acutely aware of the delicate balance between food production and world population, a fundamental understanding of germination is essential for maximum crop production (Matthews, 2002).

The constant increase of bacterial infections and their continuous emergence of drug resistant agents enhanced the idea of this study to know in-vitro anti-microbial activities of *P. farcta*. The current study also aimed to determine the allelopathic effects of *P. farcta* fruit extracts by examining *Triticum aestivum* seed germination indices.

2. MATERIALS AND METHODS

2.1 Plant description

Prosopis farcta is a simple food in Asia and the Middle East and an herbaceous plant that is difficult to control. *P. farcta* is a small tree with a height of 0.3 and 1 meter (Figure 1). When there are no weed-control measures in place, this plant can grow to 3-5 meters, which is the height of grape trees and citrus fruits. Its sophisticated root system and its growing underground stems can extend 17 to 25 meters deep into the soil, resemble to rose trees. The leaves are composite, 1.8-3.0 cm long and 8-12 pairs of small written letters folded in a short book. On each race track, there is a single brood or a long wide ring, and as it matures, it turns a very dark brown (Dheeb *et al.*, 2020).

The scientific classification of *P. farcta* is presented in (Table 1).

Table 1: *Prosopis farcta* scientific classification.

Kingdom:	Plantae
Order:	Fabales
Family:	Fabaceae
Subfamily:	Caesalpinioideae
Genus:	<i>Prosopis</i>
Species:	<i>Farcta</i>
Synonyms:	(Banks and Sol.) J.F.Macbr.



Figure 1: *Prosopis farcta* flowers and fruits.

2.2 Sample collection and preparation

P. farcta fruits were collected in Ballakayaty region in Kurdistan of Iraq. The identification of the plant was by senior taxonomist: Dr Abdullah Shakur Sardar, Salahaddin University, College of Education, Biology Department, Erbil, Iraq. The fresh fruits were thoroughly washed with distilled water for several times and then dried at room temperature. The following step involved boiling of 2.5 g of fruit in 100 mL of deionized water for 30 minutes. Then the prepared suspension was filtered through Whatman No. 40 filter paper. After filtration, a rotatory evaporator was used to evaporate the solvent. About 0.2 g of dried extract was added to 20 ml of water and placed in an ultrasound cleaner for 30 minutes. Finally the following concentrations were prepared: 2048, 1024, 512, 256, and 128 µg/ml in distilled water (Salari *et al.*, 2019).

2.3 Antimicrobial activity of *P. farcta* using well diffusion method

Sterile Petri dishes are aseptically poured with about 20 mL of Muller–Hinton before culturing. After culturing the studied organisms, 6 mm-diameter wells were bored on the agar plates using cork-borer (Khashan *et al.*, 2018). Then the holes

were filled with extracts at concentrations 2048, 1024, 512, 256, and 128 µg/ml. The plates had been incubated at 37 °C for 24 hours. After that, the outcomes were obtained by measuring the diameter of inhibition zones by millimeter. Total tests had been performed in triplicate. For confirmation Gentamycin (Gen) was used as a comparative for antibacterial activity of *P. Farcta* extracts (6 mm in diameter).

2.4 Studied bacteria

The antibacterial activity of aqueous and ethanolic extracts of *P. farcta* were tested against gram-positive bacteria *Staphylococcus aureus* and *S. aureus* ATCC25923, and gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *E. coli* ATCC 25922, *Acinetobacter baumannii* and *Klebsiella pneumonia*. All bacteria have been obtained from microbiology laboratory at Salahaddin University, College of Education, Department of Biology, Iraq.

2.5 Determination of Minimum inhibitory concentration (MIC)

For *P. Farcta* aqueous extract the concentrations (512, 256, 128 and 64 µg/ml) were used. The antimicrobial activity was conducted by determining the lowest concentration of the extract that inhibited visible growth completely and recorded as minimum inhibitory concentration (MIC) value. The negative control contained nutrient broth only, while the positive control comprised of nutrient broth and the test organisms. ELISA Biotech 800 TS (USA) was used for reading the optical density (Rotilie *et al.*, 1975). The test was conducted in triplicate (Jahromi *et al.*, 2018). Gentamycin (Gen) was used as standard antibiotics for comparing antimicrobial efficacy of *P. farcta* fruit extract and to verify the reproducibility of the results.

2.6 Germination indices evaluation

Assays of seedling growth and seed germination are a classical test system in phytotoxicity and allelopathy research (Campos *et al.*, 2019). Germination indices assessment include the following:

2.6.1 Seed germination

The percentage of seed germination was measured 10 days after seed planting. Inside separate petri plates containing absorbed filter papers with different concentrations of *P. farcta* aqueous and ethanolic extracts including (2048,

1024, 512, 256, and 128 µg/ml), 6 seeds were placed on the filter papers. For each of 10 days and at 25 °C, the filter papers have kept moist and the number of germinated seeds were recorded (Yenish and Young, 2000). Seed germination was estimated according to the equation given by Huete-Soto *et al.* (2017).

$$\% \text{ Seed germination} = \frac{\text{seeds germinated in } P.farcta \text{ extracts}}{\text{seeds germinated in control}} \times 100 \dots\dots (1)$$

2.6.2 Difference from control (DFC %)

The percentage of difference from control (% DFC) for germination was calculated from the formula of Mhatre and Chaphekar (1982) given by Mishra and Choudhuri (1999):

$$\% \text{ DFC} = \frac{\% \text{ germination of control} - \% \text{ germination of } P.farcta \text{ extracts}}{\% \text{ germination of control}} \times 100 \dots\dots (2)$$

2.6.3 % Root elongation

The percentage of root elongation was calculated from the equation given by Rai *et al.* (2014) as described in Mawlood (2020).

$$\% \text{ Root elongation} = \frac{\text{Mean root length of seeds in } P.farcta \text{ extracts}}{\text{Mean root length of control seed}} \times 100 \dots\dots (3)$$

2.6.4 Germination index (GI)

Germination index (GI) was calculated from the equation given by Rai *et al.* (2014) as described in Mawlood (2020).

$$\text{Germination index (GI)} = \frac{\% \text{ seed germination} \times \% \text{ root elongation}}{100} \dots\dots (4)$$

2.6.5 Seedling Vigor index

The Vigor index values were computed, adopting the procedure of Abdul-Baki and Anderson (1973) given by (Tizazu *et al.*, 2019).

$$\text{Seedling Vigor index} = \text{germination (\%)} \times \text{total seedling length} \dots\dots (5)$$

2.7 Statistical Analysis

Microsoft excel 2019 and SPSS version 26 software were used to analyze the data. A one-way ANOVA combined with Duncan test was used to determine the statistical significance of the germination data. A *P*-value of < 0.05 was considered a significant.

3. RESULTS AND DISCUSSION

3.1 MIC and antimicrobial activity of *P. farcta*

Results of minimum inhibitory concentrations are represented in table (2).

Table 2: Antimicrobial activity test results for both aqueous and ethanolic extracts of *P. farcta* against some pathogenic bacteria: Inhibition zone diameter (mm).

Micro-organisms	Type of <i>P. farcta</i> extract	Concentrations (µg/ml)				Positive control
		512	256	128	64	
		Optical densities				
<i>S. aureus</i>	AE	0	0.001	0.002	0.001	0.681
	EE	0.001	0.007	0.007	0.005	
<i>S. aureus</i> ATCC25923	AE	0.002	0.004	0.026	0.002	0.597
	EE	0.032	0	0	0	
<i>E. coli</i>	AE	0.028	0.032	0.034	0.022	0.703
	EE	0.008	0	0.004	0.006	
<i>E. coli</i> ATCC25922	AE	0	0.003	0.002	0.005	0.895
	EE	0.018	0.004	0.001	0	
<i>A. baumannii</i>	AE	0.013	0	0.003	0.002	0.701
	EE	0.778	0.002	0.006	0.012	
<i>A. baumannii</i> ATCC19608	AE	0.012	0	0.003	0	1.204
	EE	0.006	0.002	0.005	0.007	
<i>K. pneumonia</i>	AE	0.042	0.002	0.002	0.002	1.008
	EE	0.028	0.001	0.006	0.003	
AE: Aqueous extract EE: Ethanolic extract						

AE: Aqueous extract

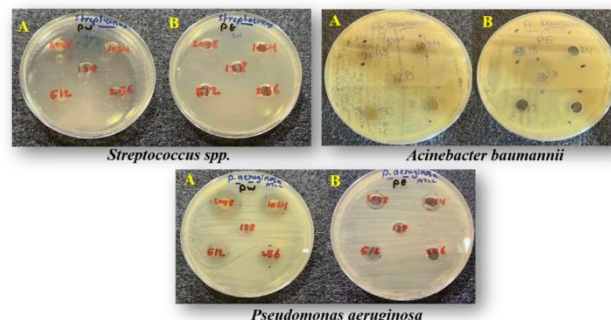
EE: Ethanolic extract

The MICs for *P. farcta* aqueous extract were 512 µg/ml against *S. aureus* ATCC25923, 256 µg/ml against *E. coli* ATCC25922, *S. aureus*, *E. coli* and *K. pneumonia* isolates, 64 µg/ml against both *A. baumannii* ATCC19608 and *A. baumannii* isolates. While the minimum inhibitory concentrations for *P. farcta* ethanolic extract were 64 µg/ml against *S. aureus* ATCC25923, 512 µg/ml against *S. aureus* isolate, 128 µg/ml against *E. coli* ATCC25922, and 256 µg/ml against *A. baumannii* ATCC19608, *E. coli*, *K. pneumonia* and *A. baumannii* isolates. However, in a previous study conducted by (Darogha, 2009) the potential antibacterial capacity of aqueous and ethanolic extract of *P. farcta* against methicillin-resistant *S. aureus* was determined. As a result, she found that the minimum inhibitory concentration (MIC) of aqueous extract was 100 mg/ml, while the MIC of ethanolic extract was 25 mg/ml, so these results were differed from those obtained in the current study. Moreover, the results are similar to the other studies conducted by (Noroozi *et al.*, 2019) and (Aldoweriej *et al.*, 2016).

The diameters of inhibition zones of both aqueous and ethanolic extracts of *P. farcta* are presented in (Table 3 and Figure 2). Results of *P. farcta* aqueous extracts against *S. epidermidis* showed inhibition zones of (23, 22, 19, 18, 13 mm) in the concentrations 2048, 1024, 512, 256 and 128 µg/ml consequently, and the inhibition zones for the *P. farcta* ethanolic extracts against this bacterium were (14, 12, 0, 0 and 0 mm) in the concentrations 2048, 1024, 512, 256 and 128 µg/ml respectively indicating the greater effects of ethanolic extracts on these bacteria.

Table 3: Antimicrobial activity of both aqueous and ethanolic extracts of *P. farcta* against some pathogenic bacteria.

Microorganisms	Type of <i>P. farcta</i> extract	Concentrations (µg/ml)				
		2048	1024	512	256	128
		Inhibition zone (mm)				
<i>S. epidermidis</i>	AE	23	22	19	18	13
	EE	14	12	0	0	0
<i>P. aeruginosa</i>	AE	21	16	14	14	0
	EE	13	12	12	0	0
<i>A. baumannii</i>	AE	15	20	0	21	0
	EE	23	25	0	21	24

A: aqueous extracts of *P. farcta*; B: ethanolic extracts of *P. farcta*.**Figure 2:** Inhibition zone diameters (mm) for the studied bacteria

Moreover, *P. farcta* aqueous extracts showed (21, 16, 14, 14 and 0 mm) as inhibition zones against *P. aeruginosa* in the concentrations 2048, 1024, 512, 256 and 128 µg/ml respectively, while the ethanolic extracts of *P. farcta* showed inhibition zones with (13, 12, 12, 0 and 0 mm) for the above described concentrations respectively. While for *A. baumannii* the concentrations 2048, 1024, 512, 256, 128 µg/ml of *P. farcta* aqueous extracts showed inhibition zones with (15, 20, 0, 21 and 0 mm) diameters respectively, and for the ethanolic extracts of *P. farcta* (23, 25, 0, 21 and 24 mm) were recorded respectively for the above-described concentrations. *P. farcta* contains varieties of alkaloids, tannins, phenols, flavonoids, terpenes, and steroids that can be obtained from its different parts, which have antibacterial capacity against gram-negative bacteria. These gram-negative bacteria are resistant to antibiotics such as minocycline, chloramphenicol, and erythromycin (Zhong *et al.*, 2022). Moreover, polyphenols are one of the most numerous and diverse group of secondary metabolites; their antioxidant properties provide the basis for antimicrobial effects. These alkaloids show good antibacterial activity by inhibiting gram-negative bacteria and gram-positive bacteria as well as fungi. Linolenic acid acts as an important anti-inflammatory agent in the body which affect microbes by: inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and biofilm formation, inhibition of

the porin on the cell membrane, alteration of the membrane (Xie *et al.*, 2015).

3.2 Germination indices

Germination is a critical stage in the life cycle of crop plants and often controls population dynamics, with major practical implications. Seed germination is the critical stage for species survival (Yang *et al.*, 2007). From the observed results (Figure 3), it has found that ethanolic extract of *P. farcta* has decreased seed germination percentage particularly in the concentration 2048 and 256 µg/ml which was 66.67 %.

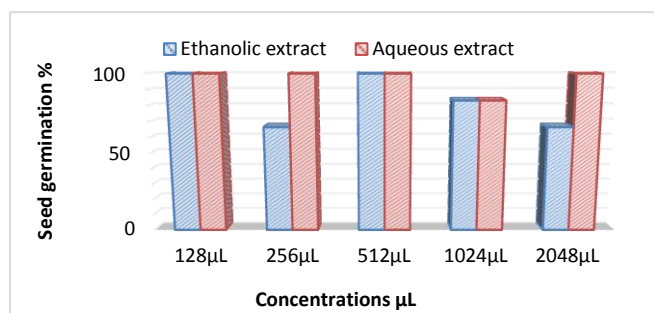


Figure 3: Seed germination % of treated *T. aestivum* seeds with both *P. farcta* aqueous and ethanolic extracts.

However, in the *P. farcta* aqueous extract, the seed germination percentages were 100 % except for the concentration 1024 µg/ml the observations of this obtained result are in contrast to Al-Barny and Beuny (2021) who found that aqueous extracts of matured fruits of *P. farcta* suppressed the germination and seedling growth of wild barley and this may refer to phenolics and tannins present in *P. farcta* fruits.

By the present experiment, plant extract concentrations have shown significant effects on seed germination when compared with control. Generally, the concentration 1024 µg/ml of *P. farcta* aqueous extract showed 20 % difference from control (Figure 4). While, *P. farcta* ethanolic extract the concentrations 2048 and 256 µg/ml have shown 50 % difference from control and 1024 µg/ml has shown 20 % difference from control.

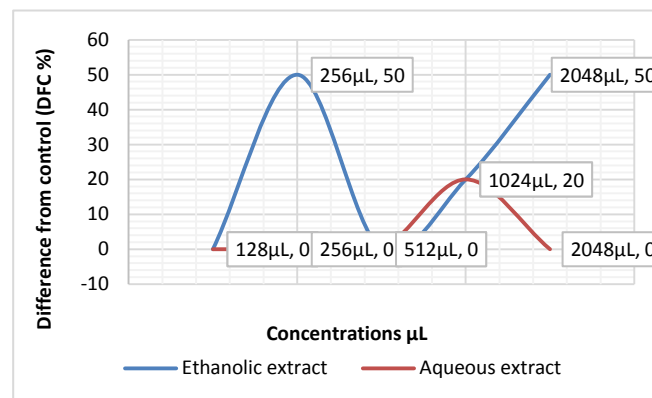


Figure 4: Difference from control (DFC %) of treated *T. aestivum* seeds with both *P. farcta* aqueous and ethanolic extracts.

Effects on root elongations are the main areas of interest in the studies of phytotoxicity (Vermeulen *et al.*, 1993). The highest percentage of root elongation (59 %) was observed in the aqueous extract of *P. farcta* 128 µL (Figure 5).

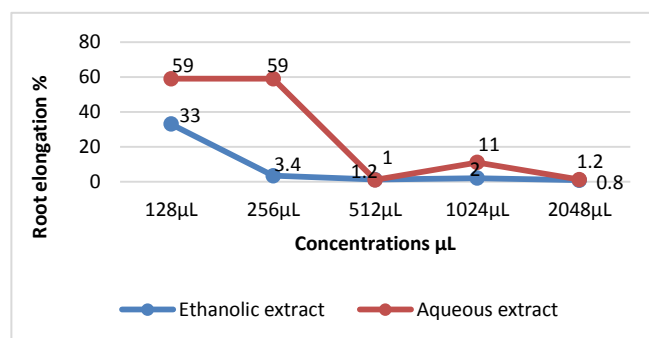


Figure 5: Root elongation % in treated *T. aestivum* seeds with both *P. farcta* aqueous and ethanolic extracts.

However, ethanolic extract of *P. farcta* has shown lower root elongation percentage (33 %), so, as the concentrations have increased, root elongation were decreased gradually. Regarding to the obtained results, Al-Barny and Beuny (2021) observed that *P. farcta* presented reducing in the germination of barley seed, increasing the average germination time and decreasing radicle and aerial parts length, so they observed tolerance index value less than 100 with the appearance of clearly phytotoxicity. These inhibitory effects may be related to the presence of allelochemicals, including tannins, wax, flavonoids and phenolic acids. Furthermore, toxicity might be due to a synergistic effect rather than the effect of any one compound or class of secondary metabolite (Nourimand *et al.*, 2011).

According to the shown data in (Figure 6), germination index values were decreased as the concentrations of *P. farcta* ethanolic extracts have increased. Whereas, 1024 µg/ml of *P. farcta* aqueous extracts showed a slight increase in germination index in comparing with 512 µg/ml of *P. farcta* aqueous extracts.

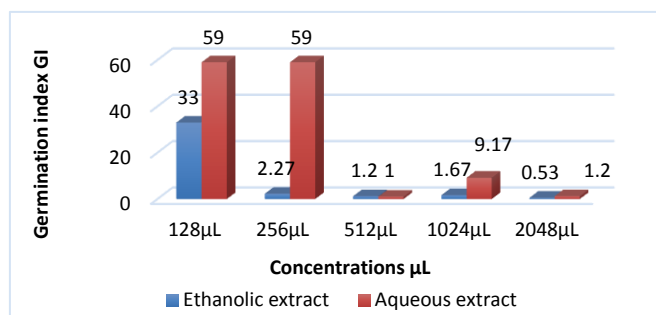


Figure 6: Germination index (GI) in treated *T. aestivum* seeds with both *P. farcta* aqueous and ethanolic extracts.

Germination process and root elongation of *T. aestivum* under both aqueous and ethanolic extracts of *P. farcta* at room temperature was active and stimulated when compared with both controls (distilled water and pure ethanol 96 %). According to (Table 4), the ethanolic extracts of *P. farcta* at 512 µg/ml has showed the best seed germination index (39.167 ± 4.902) when compared with both controls, while the lowest seed germination index (11.667 ± 4.944) was at the concentration 2048 µg/ml in comparing with both controls.

Table 4: Seed germination of *T. aestivum* under both aqueous and ethanolic extracts of *P. farcta* at room temperature.

Plant extracts		Aqueous extract	Ethanolic extract
Extract concentrations µg/ml	2048	30.833 ± 1.537^b	11.667 ± 4.944^d
	1024	34.333 ± 8.589^b	18.000 ± 4.796^c
	512	35.333 ± 7.688^b	39.167 ± 4.902^a
	256	60.667 ± 1.801^a	25.833 ± 9.075^b
	128	59.167 ± 3.005^a	31.333 ± 14.03^b
Control	Distilled water	19.167 ± 1.537^c	19.167 ± 1.537^c
	Ethanol	0.000 ± 0.000^e	0.000 ± 0.000^e

Different letters indicate significant differences among the treatments.

However, 256 µg/ml of *P. farcta* aqueous extract has showed the highest seed germination (60.667 ± 1.801) when compared with both controls and 2048 µg/ml of *P. farcta* aqueous extract showed the lowest seed germination (30.833 ± 1.537). These results are similar to the results recorded by (Azmi and Alam, 1989), but they didn't determined of plant *Prosopis* species and thus these results may be the first investigate for the first time in Kurdistan Region.

Seedling vigor index plays very crucial role in predicting the fate of any seed lot under biotic and abiotic stress conditions (Cokkizgin and Cokkizgin, 2010). Highest seedling vigor index have shown by 128 µg/ml of both ethanolic and aqueous extracts of *P. farcta* treated seeds (Figure 7). Seedling vigor index values had gradually decreased to (0.12 and 0.32) for both ethanolic and aqueous extracts of *P. farcta* treated seeds respectively. According to the observed results, ethanolic extract of *P. farcta* showed more decreasing effect on *T. sativum* seedling vigor index.

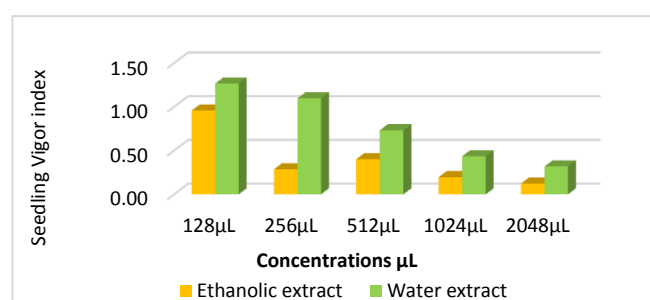


Figure 7: Seedling vigor index in treated *T. aestivum* seeds with both *P. farcta* aqueous and ethanolic extracts.

4. CONCLUSIONS

From the present study it may be concluded that aqueous and ethanolic extracts of *P. farcta* plant are active against a group of gram-positive and gram-negative bacteria particularly *Streptococcus spp.*, and this promote the using of such natural products in treatment of different kinds of bacterial infections and they also may become good synthetic drug alternatives. Another interesting result which was not concluded previously is that *P. farcta* plant extracts were affected seed germination on *T. aestivum* plant indicating the allelopathic effects of this plant.

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