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Original Article

Antibacterial effect of *Asphodelus fistulosus* aqueous and ethanolic crude extracts on gram positive and gram negative bacteria

Efeito antibacteriano de extratos brutos aquosos e etanólicos de *Asphodelus fistulosus* sobre bactérias gram-positivas e gram-negativas

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Abstract

Asphodelus fistulosus (*A. fistulosus*) is a wild plant grows in Jordan. Traditionally, it is used to treat different medical conditions and diseases such as respiratory ailments, against burns and dermatomucosal infections.This study aims to find out the effects of *A. fistulosus* aqueous and ethanolic crude extracts on *Staphylococcus aureus(S. aureus)* as gram positive bacteria and *Escherichia* coli (*E. coli)* as gram negative bacteria and to investigate which one will be affected either by aqueous and/or ethanolic crude extracts of *A. fistulosus* shooting parts that were collected from Jerash in the north of Jordan. Agar well diffusion method was used to evaluate the antibacterial activity of the crude extracts. In addition, MIC (minimum inhibitory concentration) as well as MBC (minimum bactericidal concentration) were determined against both types of bacteria. The results showed that flower aqueous extract of *A. fistulosus* was very effective against *E. coli* (20.0 ± 0.50) mm and caused a (14.0 ± 0.50) mm inhibition to *S. aureus.* The ethanolic extract of stem was very effective cauesed a (19.0 ± 0.50) mm inhibition in both bacterial species. Respectively, both *S. aureus* and *E. coli* were inhibited by ethanolic and aqueous extracts (mixture1 and mixture2) (15.0 ± 0.00 mm and 10.5 ± 0.50 mm). The highest antimbacterial activity was observed for the leaves aqueous extract against *E.coli* (0.06120 mg/mL). The obtained MIC values from *A. fistulosus* parts extracts demonstrated antibacterial activity ranged between 7.606 and 0.06120 mg/mL. The highest antimicrobial activity was recorded in the leaves aqueous extract against *E. coli*.The MBC value of stem aqueous extract was 5.00 mg/mL against both *S. aureus* and *E. coli.* On the other hand, ethanolic and aqueous extracts of the leaves gave MBC values 5.00 mg/mL, and 0.156 mg/mL, respectively, against *E. coli.*Based on the results of this study, it can be concluded that there is good inhibitory effect of aqueous and ethanolic of *A. fistulosus* shooting parts extracts on growth of *E. coli* and *S. aureus*. Adding to that, stem ethanolic extract has the most effective against *S. aureus* while aqueous extract of flower has the most effective against *E. coli*.So, it is recommended to have further future studies on the *A. fistulosus* shooting parts crude extract bioactive components and the mechanism of how these constituents affect these types of bacteria.

Keywords: *A. fistulosus*, shooting parts (flowers, stem, leaves), antimicrobial, antibacterial, gram positive, gram negative, *Escherichia coli*, *Staphylococcus aureus*, MIC, MBC.

Resumo

Asphodelus fistulosus (*A. fistulosus*) é uma planta selvagem que cresce na Jordânia. Tradicionalmente, é usada para tratar diferentes condições médicas e doenças, como doenças respiratórias, contra queimaduras e infecções dermatomucosas. Bactérias positivas e *Escherichia coli* (*E. coli*) como bactérias gram-negativas e investigar qual delas será afetada por extratos brutos aquosos e/ou etanólicos de partes de tiro de *A. fistulosus* que foram coletadas em Jerash no norte da Jordânia. O método de difusão em poço de ágar foi utilizado para avaliar a atividade antibacteriana dos extratos brutos. Além disso, MIC (concentração inibitória mínima) e MBC (concentração bactericida mínima) foram determinados contra ambos os tipos de bactérias. Os resultados mostraram que o extrato aquoso de flores de *A. fistulosus* foi muito eficaz contra *E. coli* (20,0 + 0,50 mm) e causou uma inibição (14,0 + 0,50 mm) para *S. aureus*. O extrato etanólico do caule foi muito eficaz, causando inibição (19,0 + 0,50 mm) em ambas as espécies bacterianas. Respectivamente, tanto *S. aureus* quanto *E. coli* foram inibidos pelos extratos etanólico e aquoso (mistura 1 e mistura 2) (15,0 + 0,00 mm e 10,5 + 0,50 mm). A maior atividade antibacteriana foi observada para o extrato aquoso das folhas contra *E. coli* (0,06120 mg/mL). Os valores de CIM obtidos dos extratos de partes de *A. fistulosus* demonstraram atividade antibacteriana variando entre 7,606 e 0,06120 mg/mL. A maior atividade antimicrobiana foi registrada no extrato aquoso das folhas contra *E. coli*. O valor de CBM do extrato aquoso do caule foi de 5,00 mg/mL contra *S. aureus* e *E. coli*. Por outro lado, os extratos etanólico e aquoso das folhas apresentaram valores de CBM

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de 5,00 mg/mL e 0,156 mg/mL, respectivamente, contra *E. coli*, efeito de extratos aquosos e etanólicos de partes de tiro de *A. fistulosus* no crescimento de *E. coli* e *S. aureus*. Somando-se a isso, o extrato etanólico do caule é o mais eficaz contra *S. aureus* enquanto o extrato aquoso da flor é o mais eficaz contra *E. coli*. Assim, recomenda-se a realização de estudos futuros sobre os componentes bioativos do extrato bruto de partes fotografais de *A. fistulosus* e o mecanismo de como esses constituintes afetam esses tipos de bactérias.

Palavras-chave: *A. fistulosus*, partes caulinares (flores, caule, folhas), antimicrobiano, antibacteriano, gram-positivo, gram-negativo, *Escherichia coli*, *Staphylococcus aureus*, MIC, MBC.

1. Introduction

Plants play important role in treatment of diseases when its medicinal compounds are used. Jordan is rich in valuable plant species. From 120 families and 719 genera, 2,978 species were recorded (Al-Eisawi, 1982). These numbers are always updated (Al-Eisawi, 2013). In one study of flora of Jordan Oran And Al-Eisawi (1998) reported that from the total flora of Jordan, medicinal plants account for 20%. Many studies were performed on many local medicinal plants in Jordan concerning their different roles as antioxidant and immunomodulatory adding to that their effects on inflammations, cancer and microbes (Mohammad et al., 2010; Talib And Mahasneh, 2010; Qnais et al., 2012; Zeidan et al., 2013; Obeidat et al., 2012). The traditional use of medicinal plants are common among different populations around the world. The worldwide distribution of some medicinal plants as well as their valuable effects on having effects on certain types of bacteria that cause disease to human allow people to investigate their potential benefits and think to go through different ways to study some times each species of a given plant to evaluate and test their components that could be used as a therapeutic dose for patients with no or little side effects compared with drugs. The World Health Organization (WHO) reported that about 70% of the world population currently use plants for medicinal purposes with high usage in Africa, South America and Asia (Who, 1983). Medicinal Plants status in Jordan concerning their biological effects on different living organisms and applications in folk medicine were conducted by Oran (2014).

Recently, a study on the genus *Asphodelus* was done, concerning its biological activity, found that only 30% of 18 species studied were used traditionally of these, *Asphodelus fistulosus* (*A. fistulosus*) (Malmir et al., 2018). This study reported that *A. fistulosus* is used in various countries including Palestine, Egypt, Libya, Cyprus and Spain for their effects in dermatomucosal infections. Al-Harbi (2017) reported that dermatitis is treated by the whole plant. The whole plant is used worldwide for many purposes such as treatment of ulcer, laxative, spasmogenic, diuretic and stimulant. The toothache is treated traditionally using seeds (Jafri And EL-Gadi, 1978; Agrawal, 1990). In Saudi Arabia, aqueous and methanolic leave extracts of *A. fistulosus* was studied on four types of bacteria by Sulieman et al. (2017) while Alam et al. (2018) studied antimicrobial activities of seed ethanolic extract of *A. fistulosus*. In 2009, the roots usage was reported by Leonti et al. (2009) to treat respiratory ailments, against burns and as a cutaneous disinfectant. In Saudi Arabia, Al-Rass province, use the seeds, bulk and flowers as anthelmintic as well as against wound sepsis, swellings and stomachache (El-Ghazali et al., 2010). In Jordan valley, it is used for dropsy, constipation, and ulcers also as diuretic and laxative. For ulcer and inflamed organs the seeds are applied externally while the fresh leaves are used as condiments. In addition, the seeds are eaten with yoghurt (Qasem, 2015).

Concentrations of Pb, Zn, Cu and Cd in *A. fistulosus* roots and stems were investigated in Jordan by Al-Fawwaz And Al-Khazaleh (2017). As far as we know few researchers studied antibacterial activity of *A. fistulosus* in Jordan.

The aim of the current study was to find out the effects of *A. fistulosus* aqueous and ethanolic crude extracts on Gram positive bacteria: *Staphylococcus aureus* (*S. aureus*) and Gram negative bacteria: *Escherichia coli* (*E. coli*).

2. Materials and Methods

2.1. Sample collection

A. fistulosus was harvested in February 2021 from Jerash in north of Jordan (see Figures 1A and 1B). Then

Figure 1. The area of studied *Asphodelus fistulosus* in Jerash -Jordan (A and B).

the shooting system parts (stem, leaves and flowers) were weighed individually and were carefully cut into pieces and washed with distilled water. After that, the parts were dried in oven at 60ºC for one hour and were put in aerated shade place untill it completely dried. Finally, it was ground by herb grinder to get fine powder.

2.2. Crude extracts preparation

The powder of each plant part was divided to two portions in order to prepare aqueous and ethanolic crude extracts.

2.2.1. Aqueous (water) crude extract

Plant part powder was weighted and soaked with autoclaved distilled water (1:10 weight/ volume ratio), they were boiled for 4hours at 70 °C and left to cool, after that the prepared aqueous extract was filtered using filter paper followed by filtration with 0.45um Millipore filter membrane. The solvent of liquid extract was removed completely by putting the liquid extract into rotary evaporator with vacuum pump at 40 °C, under reduced pressure of 175 mbar with rotation at the speed of 150 rpm. The crude extracts were then re-suspended in 1X phosphate buffer saline (PBS, SIGMA) to reach a concentration of 500 mg/mL and finally stored at 4 °C in glass dark bottles till used in the assay (Kim et al., 2011; Awaisheh, 2013; Abubakar And Haque, 2020).

2.2.2. Ethanolic crude extract

The plant ethanolic extract was prepared by maceration extraction method. Each plant part was weighted and soaked in (1:10 weight/ volume) ratio with 70% ethanol, after that they were put on shaker for 24hours at 150rpm. Then it was filtered using filter paper followed by filtration with 0.45um Millipore filter membrane. After that, the solvent of the liquid extract was removed completely as illustrated previously in aqueous (water) extract preparation above. Finally, 500mg/mL crude extracts were obtained when re-suspended in 1X phosphate buffer saline and stored at 4 °C in glass dark bottles till used in the assay (Abubakar And Haque, 2020; Weli et al., 2018; Chan et al., 2015).

2.3. Bacterial strains and culture conditions

Standardized pure cultures of bacterial strains procured from the Faculty of Agriculture, Department of Nutrition and Food Processing, Al-Balqa Applied University, were used.

The antibacterial potency of each plant extract was evaluated using two bacterial strains one of Gram positive (*S. aureus* ATCC 25923) and one strain of Gram negative (*E. coli* ATCC 43888) bacteria.

Prior to the experiment; three culture transfers were performed to resuscitate each culture and then they were transferred individually to Mueller Hinton broth and saline solution (0.85%) supplemented with (0.2%) Tween 80. They were grown at 37 °C for 24hours, to reach the stationary phase (Awaisheh And Ibrahim, 2009).

2.4. Screening of antimicrobial activity

Different extracts of different plant parts (flowers, stem and leaves) either aqueous or ethanolic extracts were investigated to evaluate their antibacterial activity against the bacterial strains (*S. aureus* ATCC 25923) and (*E. coli* ATCC 43888) using disc diffusion method. A mixture of the plant parts extracts were also obtained to determine its antibacterial activity against those bacterial strains by mixing the extract in (1:1:1) ratio of (flower, stem and leaves), respectively; either ethanolic or aqueous extracts (mixture 1 and mixture 2), respectively.

Eigteen-hours culture was diluted with sterile physiological saline solution 0.85% (w/v) sodium chloride supplemented with (0.2%) Tween 80 to achieve an inoculum of approximately 10^5 CFU/ mL. A volume of 100µl of bacterial inoculum was placed into the surface of pre-dried Mueller-Hinton Agar (MHA) and allowed to remain in contact until completely dried.

Antimicrobial activity of *A. fistulosus* extracts was screened using Agar well diffusion assay (AWDA). Wells (5 mm) were made on each plate, then 30µl (500mg/mL) of each crude extract alone or in combination (mixture) were added. After allowing 20 minutes at room temperature for the extract to diffuse across the surface, inoculated petri dishes were incubated at 37 °C for 24 hrs, the inhibition zone was measured in millimeter using caliper for each extract (Al-Nabulsi et al., 2014).

2.5. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC values were determined for each crude extract alone or in combination (mixture 1 and mixture 2). MIC values were determined using 96-wells micro-dilution method (Awaisheh, 2013; Yasunaka et al., 2005). 100 µl of overnight culture containing 6.0 log10 CFU/mL of each bacterium were added to corresponding wells. A two fold serial dilution of each extract was prepared by using DMSO ranging from 20mg/mL to 0.625mg/mL. Then 100 µL of each serial dilution were added into each well, so the total volume was 200 µL, the plates were then sealed and incubated at 37 °C for 24hours. Absorbance (Abs) was measured at 600 nm using microplate reader (ELX 800, Biotek, High-land Park, VT, USA). Negative controls were also prepared by using 0.05% DMSO, un-inoculated Muller Hinton broth and inoculated Muller Hinton broth with 0.05% DMSO. MBC was also determined by plating 100µL aliquots from the clear wells by pour plate technique.

3. Data Analysis

The obtained data from this research was analyzed using Prism program (version 6.1).

4. Results

4.1. Antimicrobial (antibacterial) activity

Evaluation of antibacterial activity of these plant extracts was recorded in Table 1. The results revealed that plant

part extracts were potentially effective in suppressing microbial growth of both strains *S. aureus* ATCC 25923 and *E. coli* ATCC 43888. The ethanolic extract of stem was very effective showed a (19.0 ± 0.50) mm inhibition in both strains. Both *S. aureus* and *E. coli* were inhibited by ethanolic and aqueous extracts (mixture 1 and mixture 2) (15.0 ± 0.00 and 10.5 ± 0.50), respectively. Flower aqueous extract of *A. fistulosus* was very effective against *E. coli* (20.0 ± 0.50) mm and caused a (14.0 ± 0.50) mm inhibition to *S. aureus*. Stem ethanolic extract was the most effective against *S. aureus* while aqueous extract of flower was the most effective against *E. coli*.

4.2. Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of the effective plants extract

The lowest concentration of *A. fistulosus* extracts that inhibit the bacterial growth after 24 hours of incubation is illustrated in Table 2 and Figures 2-13. The MIC values obtained from *A. fistulosus* parts extracts demonstrated antibacterial activity ranged between 7.606 and 0.06120 mg/mL.

Ethanolic and aqueous extracts of the flower part of plant gave MIC values of, 2.492 mg/mL, and 2.830 mg/mL, respectively against *S. aureus*, 0.0779 mg/mL and 1.503 mg/mL respectively against *E. coli*. On the other hand, the highest antimicrobial activity was observed for the leaves aqueous extract against *E. coli* (0.06120 mg/mL).

Table 3 demonstrates the lowest extract concentration killing 99.9% of the bacterial inocula after 24 h incubation at 37 °C for both ethanolic and aqueous extracts. Ethanolic and aqueous extracts of the leaves gave MBC values of, 5.00 mg/mL, and 10.00 mg/mL against *S. aureus* respectively, and 5.00 mg/mL, and 0.156 mg/mL against *E. coli* respectively. On the other hand, the MBC value of stem aqueous extract was 5.00 mg/mL against both *S. aureus* and *E. coli*.

5. Discussion

In addition to other four species of the genus *Asphodelus*, *A. fistulosus* has been reported to have traditional uses (Malmir et al., 2018). Phytochemically, many compounds are found in it such as phenolic acids, triterpenoids, flavonoids and anthraquinones (Malmir et al., 2018). *A. fistulosus* antimicrobial activity of seeds ethanolic crude extract and various fraction showed moderate activity against the gram positive and gram negative bacterial strains (Alam et al.,

Table 1. Antibacterial activity of ethanolic and aqueous plant extracts against the clinical isolates (bacterial strains)†, inhibitory zone in millimeter (mm) measured by caliper.

Extract / Microorganism	Ethanolic extract			Aqueous (water) extract			mixture 1*	mixture 2**
	Flower	Leaves	Stem	Leaves	Stem	Flower		
Staphylococcus aureus	$15.5^{a(b)1,2,3} \pm 0.50$	$6.5^{b(h)} \pm 0.50$	$19.0^{a(a)} \pm 0.50$	$7.0^{a(g)} \pm 0.50$	$9.5^{a(f)} \pm 0.50$	$14.0^{b(d)} \pm 0.50$	$14.0^{b(de)} \pm 0.50$	$15.0^{a(c)} \pm 0.00$
Escherichia coli	$3.0^{b(h)} \pm 0.00$	$10.0^{a(de)} \pm 0.00$	$19.0^{a(b)} \pm 0.50$	$6.5^{b(f)} \pm 0.50$	$4.0^{b(g)} \pm 0.00$	$20.0^{a(a)} \pm 0.00$	$15.5^{a(c)} \pm 0.50$	$10.5^{b(d)} \pm 0.5$

[1]Results are Mean ± standard error of mean (SEM) of two determinations of two independent experiments; [2]Results with different letters in brackets in the same row are significantly different ($p<0.05$); [3]Results with different letters in the same column are significantly different ($p<0.05$); *Plant ethanolic extract mixture from flower, leaves and stem mixed in (1:1:1) ratio; **Plant aqueous extract mixture from flower, leaves and stem mixed in (1:1:1) ratio; †(*S. aureus* ATCC 25923) and (*E. coli* ATCC 43888) bacteria.

Table 2. MIC* values (mg/mL) of different extracts used against different bacterial strains†.

Extract / Microorganism	Ethanolic extract			Aqueous (water) extract		
	Flower	Leaves	Stem	Leaves	Stem	Flower
Staphylococcus aureus[1]	2.492 ± 0.00	2.210 ± 0.00	0.6651 ± 0.00	7.606 ± 0.00	4.167 ± 0.00	2.830 ± 0.00
Escherichia coli[1]	0.0779 ± 0.00	4.040 ± 0.00	5.384 ± 0.00	0.0612 ± 0.00	2.510 ± 0.00	1.503 ± 0.00

*Minimum Inhibitory Concentration; †(*S. aureus* ATCC 25923) and (*E. coli* ATCC 43888) bacteria; [1]Microbial strains with MIC values.

Table 3. MBC* values (mg/mL) of different extracts used against different bacterial strains†.

Extract / Microorganisms	Ethanolic extract			Aqueous (water) extract		
	Flower	Leaves	Stem	Leaves	Stem	Flower
Staphylococcus aureus[1]	5.000 ± 0.00	5.000 ± 0.00	1.250 ± 0.00	10.00 ± 0.00	5.000 ± 0.00	5.000 ± 0.00
Escherichia coli[1]	0.156 ± 0.00	5.000 ± 0.00	10.00 ± 0.00	0.156 ± 0.00	5.000 ± 0.00	2.500 ± 0.00

*Minimum Bactericidal Concentration; †(*S. aureus* ATCC 25923) and (*E. coli* ATCC 43888) bacteria; [1]Microbial strains with MBC values.

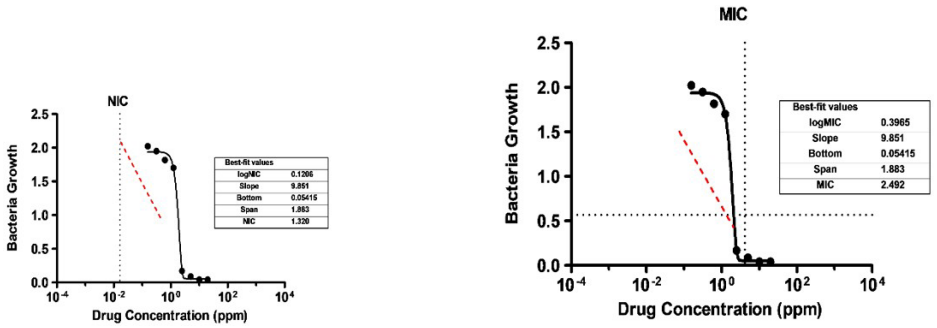

Figure 2. MIC of flower ethanolic extract against *S. aureus* (*S. aureus* ATCC 25923). NIC = Non Inhibitory Concentration.

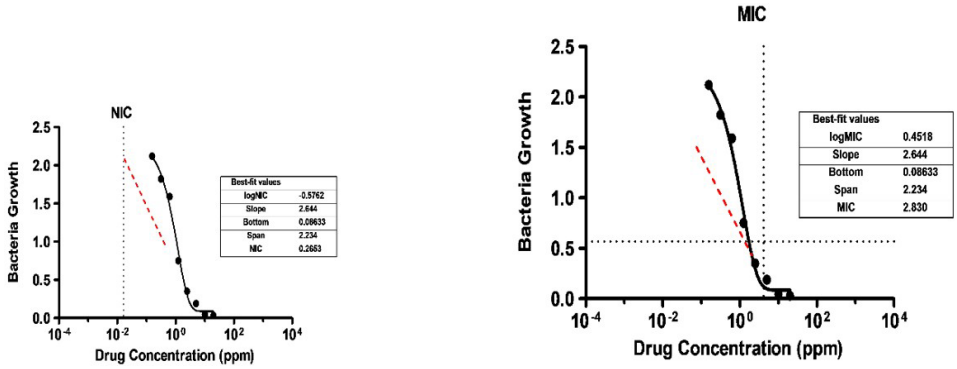

Figure 3. MIC of flower aqueous extract against *S. aureus* (*S. aureus* ATCC 25923).

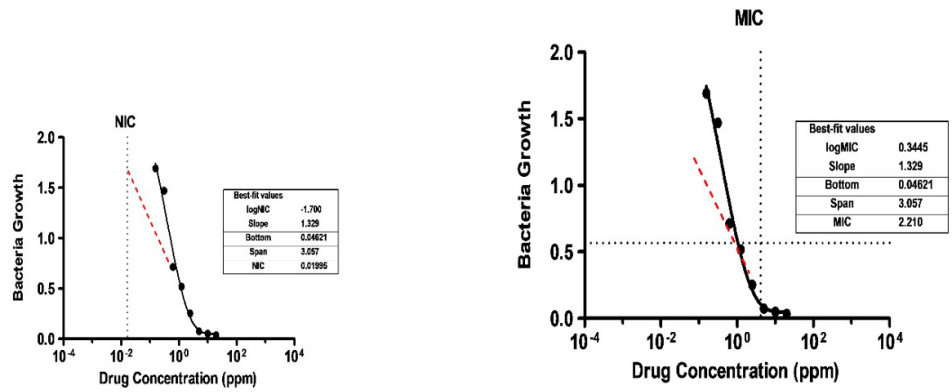

Figure 4. MIC of leaves ethanolic extract against *S. aureus* (*S. aureus* ATCC 25923).

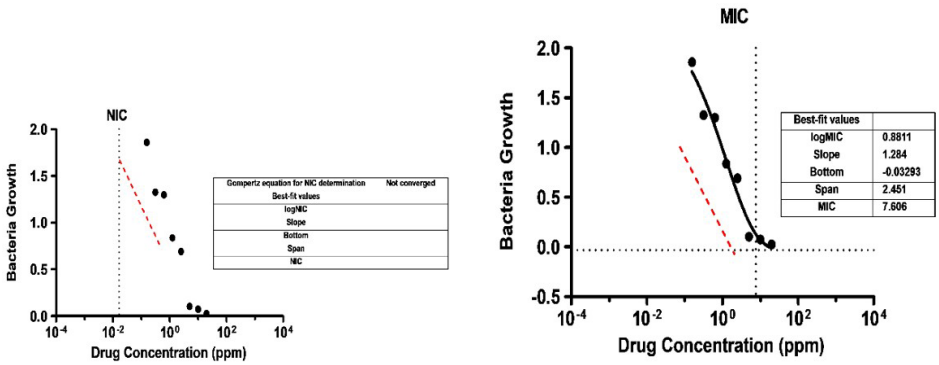

Figure 5. MIC of leaves aqueous extract against *S. aureus* (*S. aureus* ATCC 25923).

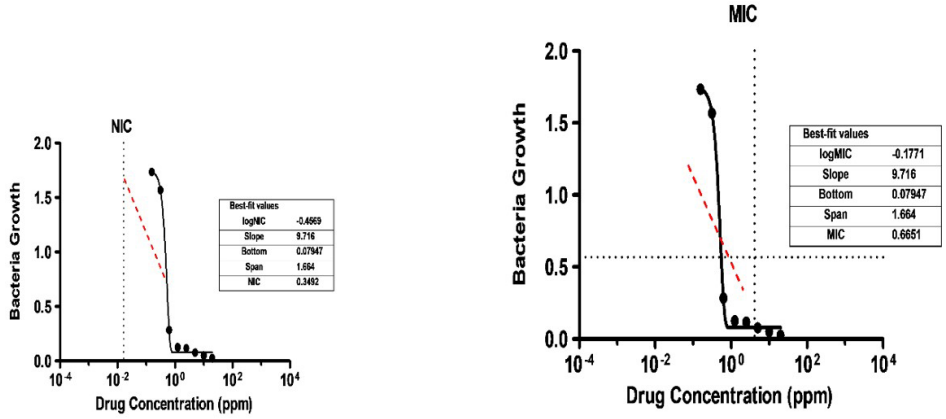

Figure 6. MIC of stem ethanolic extract against *S. aureus* (*S. aureus* ATCC 25923).

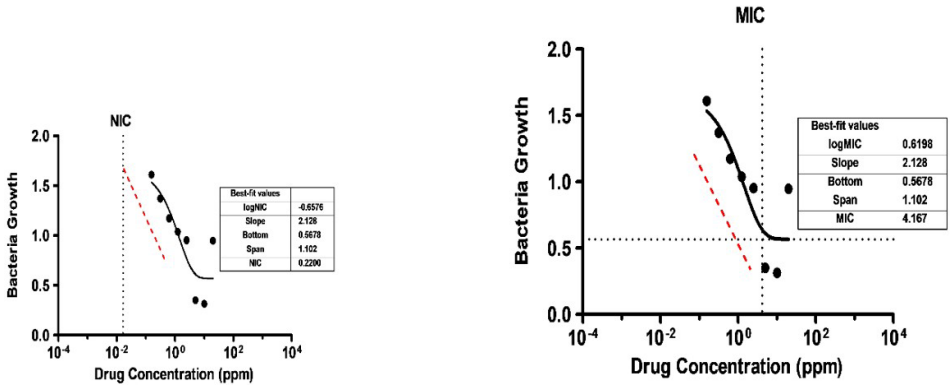

Figure 7. MIC of stem aqueous extract against *S. aureus* (*S. aureus* ATCC 25923).

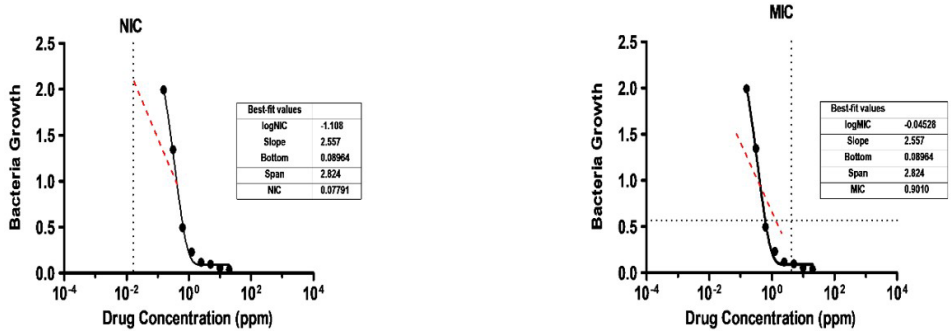

Figure 8. MIC of flower ethanolic extract against *E. coli* (*E. coli* ATCC 43888).

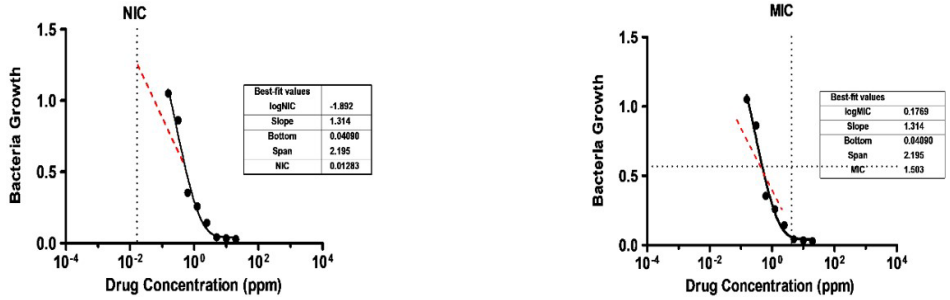

Figure 9. MIC of flower aqueous extract against *E. coli* (*E. coli* ATCC 43888).

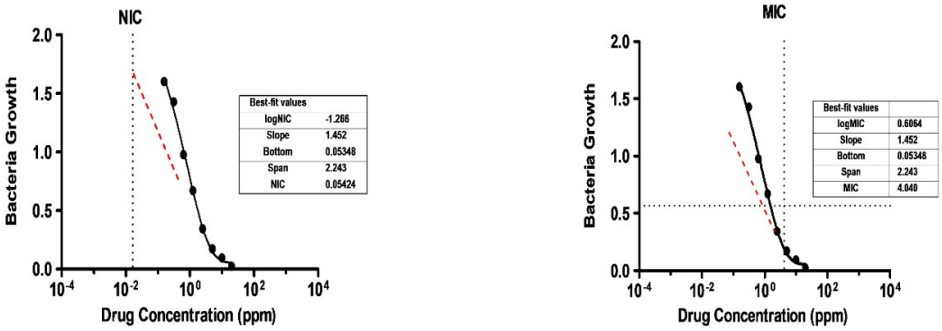

Figure 10. MIC of leaves ethanolic extract against *E. coli* (*E. coli* ATCC 43888).

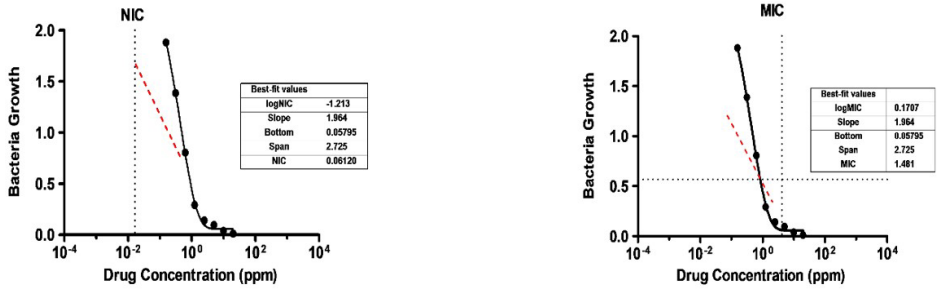

Figure 11. MIC of leaves aqueous extract against *E. coli* (*E. coli* ATCC 43888).

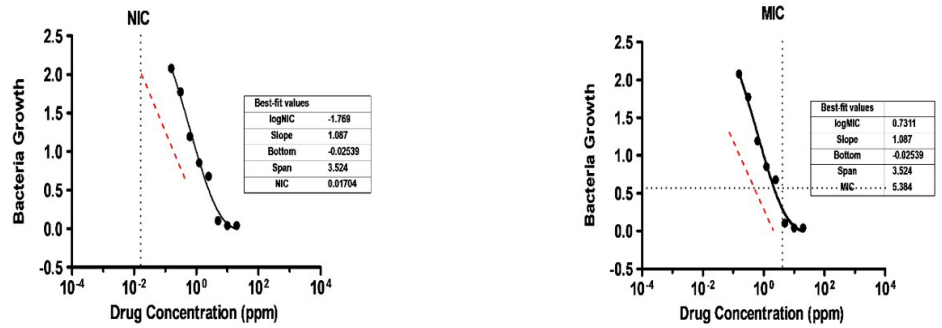

Figure 12. MIC of stem ethanolic extract against *E. coli* (*E. coli* ATCC 43888).

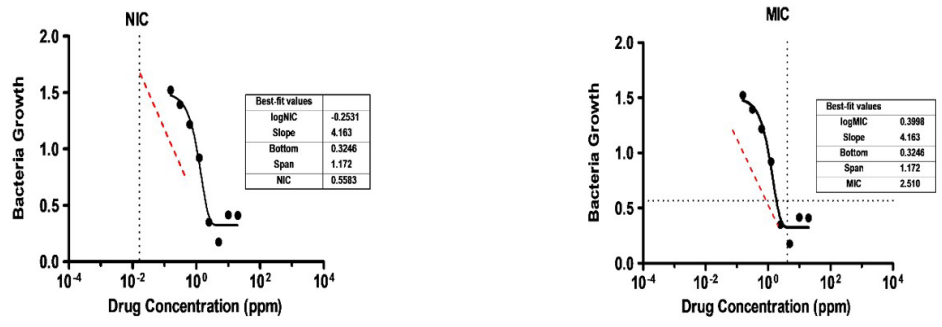

Figure 13. MIC of stem aqueous extract against *E. coli* (*E. coli* ATCC 43888).

2018). The ethanolic crude extract was more active against *E. coli* showing zone of inhibition of 16 (500 µg/disc) and 10 mm (250 µg/ disc) for *E. coli.*

To the best of our knowledge no study has been done on ethanolic as well as aqueous extracts of *A. fistulosus* shooting parts (leaves, stem and flowers) effects on Gram positive and Gram negative bacteria especially *S. aureus* and *E. coli.* in Jordan and few studies were done around the world (Malmir et al., 2018). Leaves water extract of *A. fistulosus* did not inhibit growth of *E. coli* while the methanolic extract of the same plant part did (Sulieman et al., 2017)

On the other hand, other species of the same genus were studied in different countries around the world. For example, in 2013 antimicrobial activity of *A. tenuifolius* (cav.) against *S. aureus* was founded by Dangi et al. (2013). Khalfaoui et al. (2021) studied the aerial parts of *A. tenuifolius* and they found that organic (chloroformic) extracts had antibacterial effects against both Gram-positive and -negative bacteria with MBC/MIC ratio showed that it is bacteriostatic. In another study good antibacterial effect against *E. coli* was reported when methanol was used to prepare exctract from all parts (flowers, shoot and root) of *A. tenuifolius* together (Ahmed et al., 2016).

The results of ethanolic extract of *A. microcarpus* leaves revealed that Gram-positive bacteria have higher inhibition when compared with the Gram-negative bacteria *E. coli* and *E. coli* has the lowest value (Petrillo et al., 2017).

6. Conclusion

The effective use of *A. fistulosus* as medicinal plant is supported scientifically by the obtained results in this work. In addition, it can be concluded that *A. fistulosus* shooting parts have potent antibacterial activity. The results could provide a basis for developing suitable doses medication to treat gram positive and/or gram negative bacteria that cause disease to human.

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