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Research Paper

Antibacterial activity of leaf extracts of Silene succulent Forsk. (Caryophyllaceae) against clinically important bacteria

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ABSTRACT

The in vitro antibacterial activity of *Silene Succulent* leaves extracts of water, ethanol, methanol and chloroform against five species of clinical pathogenic bacteria was assessed. The antibacterial activity was tested by the agar well diffusion method. The organic extracts showed variable results for the tested bacterial species, the most effective organic extract was chloroform extract and exhibited an inhibitory effect against four bacterial species at all concentrations used *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella sp*. and *Escherichia coli*, while the aqueous extract was effective against two bacterial species at all concentrations used *S.aureus* and *E.coli*. The organic extracts were the most effective extracts and showed bacteriostatic activities against the highly susceptible species of pathogenic bacteria *S. aureus* and *P. aeruginosa* with MIC ranged from 12.5 to 25 mg/ml except *P. aeruginosa* which was less sensitive to ethanol extract and its MIC reached to 50 mg/ml. These plant extracts which proved to be potentially effective can be used as natural alternative preventives to control diseases caused by this bacterial species and avoiding health hazards of chemically antimicrobial agent applications.

Key words: *Silene succulent* Forsk, antibacterial activity, agar well diffusion method, MIC.

INTRODUCTION

Antibiotics provide the basis for fungal and bacterial infections therapy. The discovery of antibiotics and making use of them as chemotherapeutic agents has made the medical fraternity to believe that they will eradicate various infectious diseases. However, indiscriminate use of antibiotics in human and veterinary healthcare systems has lead to the emergence of multi-drug resistant (MDR) strains of different groups of microorganisms (Harbottle et al., 2006; Alam et al., 2013). The emergence and dissemination of MDR bacteria have made chemically synthesized antibiotics ineffective for the treatment of infectious diseases caused by such bacteria (Amador et al., 2009). These circumstances have propelled the researchers and scientists to explore new antimicrobial substances from various sources such as medicinal plants (Cordell, 2000). Medicinal plants represent a rich source of antimicrobial agents (Chandra, 2013). A wide range of medicinal plant extracts is used to treat several infections as they have potential antimicrobial activity. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (Hamburger and Hostettmann, 1991). Thus, they have been used in the treatment of various human diseases for thousands of years all over the world. Similarly, some plants have been used by rural people in Libya for the treatment of several diseases. Several studies have established that many plants contain substances like peptides, tannins, alkaloids, essential oils, phenols and flavonoids among others, which have antimicrobial properties (Arora and Kaur, 1999; Okigbo and Omodamiro, 2006). The genus *Silene* (family Caryophyllaceae) comprises more than 700 species of annuals, biennials and perennials which are mainly distributed in temperate zones of the northern hemisphere of Eurasia and America but also in Africa (Mabberley, 2008;

Greuter 1995).

In Libyan flora there are some species of this genus, according to some field studies that were conducted in Libya, among these species is the species tested in this study (*Silene succulent*). In the most recent taxonomic revision covering the entire genus, *Silene* has been divided into 44 sections (Chowdhuri, 1957). The genus consists mainly of herbaceous plants and more rarely, small shrubs or subshrubs, the flowers have free petals, with each petal consisting of a usually visible limb that can be divided or entire and a claw that is included within the synsepalous calyx (Nilufar et al., 2014). Most of its species are hermaphrodite and a very few numbers of its species are dioecious or gynodioecious (Greuter, 1995). *Silene* produces a diversity of secondary metabolites, many of them are important for the plants as defense compounds against herbivores and microbes (Wink, 2010; Wink, 2011). Chemical compounds in some species of this genus contain ecdysteroids, Phyto-ecdysteroids, flavonoids, saponins and triterpenes (Mamadalieva, 2012), lipids (triglycerides, free fatty acids, free sterols), benzenoid compounds (phenylacetaldehyde), volatile monoterpenes alkenes (α-pinene, β-pinene, limonene), vitamins, organic acids, microelements, amino acids (Mamadalieva, 2012; Karamian and Ghasemiou, 2013). Several *Silene* species have been used in traditional medicine to treat inflammations, bronchitis, cold, and infections or as a diuretic, antipyretic, analgesic and emetic (Ali et al., 1999; Hirst, 2005). This study, it is aimed to specify the antimicrobial activity of leaf extracts of *Silene succulent* against selected human pathogenic bacteria.

MATERIALS AND METHODS

Collection and preparation of plant

Fresh samples were collected from the leaves of the *S.succulent* in the middle of the spring month of 2020 from the Zueitina region, northwest of Benghazi in Libya the leaves were placed in plastic bags. Leaves were washed gently under tap water to remove the dust and left in the air under shade to dry for 2 weeks then it was cut into small pieces. The leaves were then crushed into powder using an electric blender (condux type), transferred into a glass container and preserved until the extraction procedure was performed in the laboratory.

Preparation of extracts

According to the method of Sukanya et al. (2009) with some modifications, 50gm of the powder of *S. succulent* were filled in the thimble and extracted successively with 250 ml each of sterile distilled water, ethanol, methanol and chloroform using a Soxhlet extractor for 24 h. All the extracts were evaporated using a rotary evaporator. At last, 1.9 g of dried extracts were obtained, all the extracts were dissolved in dimethyl sulfoxide (DMSO) (Perez et al., 1990). Three concentrations of extracts were prepared, which are 50, 100 and 150 mg/ml and stored at 4°C in airtight bottles until further use.

Test bacteria

Bacterial species were obtained from the microbiology laboratory of Benghazi Medical Centre (BMC), Benghazi in Libya. In total, five microorganisms were tested against the above-mentioned plant extracts in which two were Gram-positive *S. aureus* and *E. faecalis,* three were Gram-negative namely *E. coli, Klebsiella sp.* and *P. aeruginosa.* The species were maintained on nutrient agar slants at 4°C.

Preparation of culture media

Mueller Hinton Agar (MHA) media was prepared by suspending 38 g in 1000 ml of distilled water heat to boiling to dissolve the medium completely. The media was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile petri plates.

Preparation of the bacterial suspension

Bacteria stock cultures *S. aureus, E. faecalis, P. aeruginosa, E. coli,* and *Klebsiella sp.* were sub-cultured onto Blood Agar (BA) plates and incubated overnight at 37°C. The next day, three to four discrete bacterial colonies with similar morphology were inoculated into 10 ml sterile Mueller Hinton broth (MHB) and incubated overnight at 37°C. The overnight bacterial suspensions were adjusted to 0.5 McFarland Standard with sterile MHB broth, approximately1.5×106 cell/ml. To aid comparison, the adjustment of bacterial suspensions to the density of the 0.5 McFarland Standard was done against a white background with contrasting black lines (Teh et al., 2017).

Antibacterial activity assay

Antibacterial activity of the aqueous extract and organic extracts of the plant sample was evaluated by the agar well diffusion method (Perez et al., 1990)0. Gentamycin was used as the standard antibacterial agents. Petri plates were poured with MHA media and allowed to solidify to make a base layer. The bacterial suspension of each test was evenly spread over the media by sterile cotton swabs. The plates have been kept to dry and a sterile cork borer (7mm in diameter) was then used to punch wells (four wells) in the

Table 1: Zones of inhibition of leaf extracts of S. succulent against pathogenic bacterial species

		The diameter of inhibition zone of bacteria (mm)														
		Concentration in mg/ml														
		Gram (+ve) pathogenic bacteria						**Gram (-ve) pathogenic bacteria**								
S.No.	**Bacterial sp.**	***S.aureus***			***E. faecalis***			***P.aeruginosa***			***Klebsiella sp.***			***E.coli***		
1	Plant extract	**50**	**100**	**150**	**50**	**100**	**150**	**50**	**100**	150	50	100	**150**	50	100	150
2	Aqueous extract	12	12	14	**-**	**-**	**-**	-	-	-	**-**	-	-	9	11	12
3	Ethanol extract	11	12	15	**-**	**-**	**-**	**13**	14	15	**-**	**-**	**-**	**-**	**8**	**11**
4	Methanol extract	13	14	16	**-**	**9**	**12**	8	9	11	**-**	**-**	**-**	-	-	-
5	Chloroform extract	12	14	**17**	-	-	-	12	14	**16**	**-**	**14**	**15**	**8**	**10**	**14**
6	Gentamycin	20	23	25	16	**19**	**21**	**12**	**14**	**15**	**19**	**21**	**24**	**25**	**27**	**30**
7	DMSO (control)	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
		No activity (there was no zone of inhibition)														

Table 2: MIC of the most effective plant extracts against *S. aureus* and P. aeruginosa.

		The diameter of inhibition zone of bacteria (mm)											
		Concentration in mg/ml											
		Gram (+ve) pathogenic bacteria						**Gram (-ve) pathogenic bacteria**					
S.No	**Bacterial sp.**	***S.aureus***						***P.aeruginosa***					
	Plant extracts	6.25	12.5	25	50	100	200	6.25	12.5	25	50	100	200
	Ethanol extract	**-**	**9**	**9**	11	13	**16**	**-**	**-**	**-**	**12**	**14**	**16**
	Methanol extract	**-**	10	13	13	**14**	17	**-**	**-**	**7**	**8**	**9**	**11**
	Chloroform extract	--						No activity (there was no zone of inhibition)					

agar medium. Subsequently, wells were filled with 100µl of each extract at various concentrations of 50, 100, and 150 mg/ml and allowed to diffuse at room temperature for 30 minutes. Finally, the plates were incubated at 37°C for 24 h. The solvent DMSO that would not inhibit the growth of the microorganisms (Zgoda and Porter, 2001) was used as the negative control under the same condition for each bacterial species. The resulting diameters of zones of inhibition, including the diameter of the well, were measured using a ruler and reported in millimeter. To maintain the consistency of measurements, each zone of inhibition was measured twice (one vertical and one horizontal measurement) and the average value was taken. The experiment was performed in triplicate for each bacterium and plant extract and the mean zone of inhibition was calculated for each crude extract and standard antibiotic.

Determination of minimum inhibitory concentration (MIC)

The MIC test was prepared according to the method of Mostafa et al. (2018) with some modifications. MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h of incubation. The most effective plant extracts which exhibits a strong antibacterial activity at 150 mg/ml were tested to determine their MIC using a well diffusion method and evaluate their efficiency in controlling bacterial species causing diseases. Different concentrations of the effective plant extracts (6.25, 12.5, 25, 50, 100 and 200 mg/ml). A sterile cork borer (6mm in diameter) was then used to punch wells (six wells) in the agar medium. Subsequently, wells were filled with 100µl of each different concentration of the effective plant extracts. Mueller-Hilton agar was poured into sterile petri dishes and seeded with bacterial suspensions of the pathogenic species. The plates were kept in the fridge at 5°C for 2 h. then incubated at 37°C for 24 h. The inhibition zones were measured by a ruler.

RESULTS

S.succulent plant was investigated to evaluate their antibacterial activity against some pathogenic bacterial species (Table 1) including two species of Gram positive bacteria (*S. aureus* and *E. faecalis* and three species of Gram negative bacteria *P. aeruginosa, Klebsiella sp*. and *E. coli* using well diffusion method with three various concentrations of 50, 100, and 150 mg/ml. The DMSO solvent is used as a negative control and the Gentamycin is used as a positive standard. Evaluation of antibacterial activity of these plant extracts was recorded in Table 2 and Figures 1-7. In this study, the plant extracts showed significant antagonistic activity against most tested bacteria species. The aqueous extract substantially inhibited the growth of the bacteria *S.aureus* and *E.coli* but show no inhibitory effect against *E. faecalis, P. aeruginosa,* and

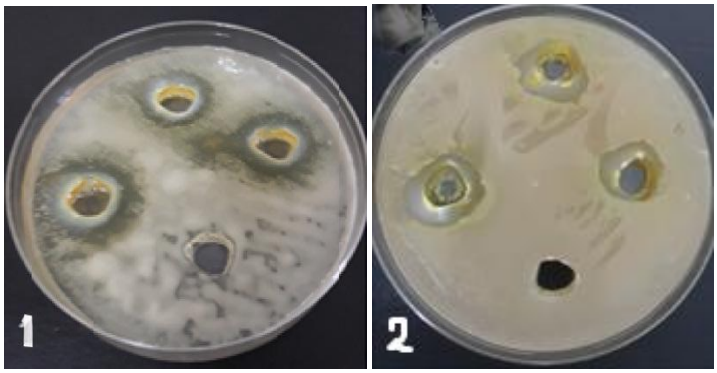

Figure 1: Effect of aqueous extract against 1- S.aureus and 2-*E.coli* at 50,100 and 150 mg/ml concentrations, respectively (from right to left) compared with control.

Figure 2: Effect of ethanol extract against 1- S.aureus, 2- P.aeruginosa and 3-E.coli at 50,100 and 150 mg/ml concentrations, respectively (from right to left) compared with control.

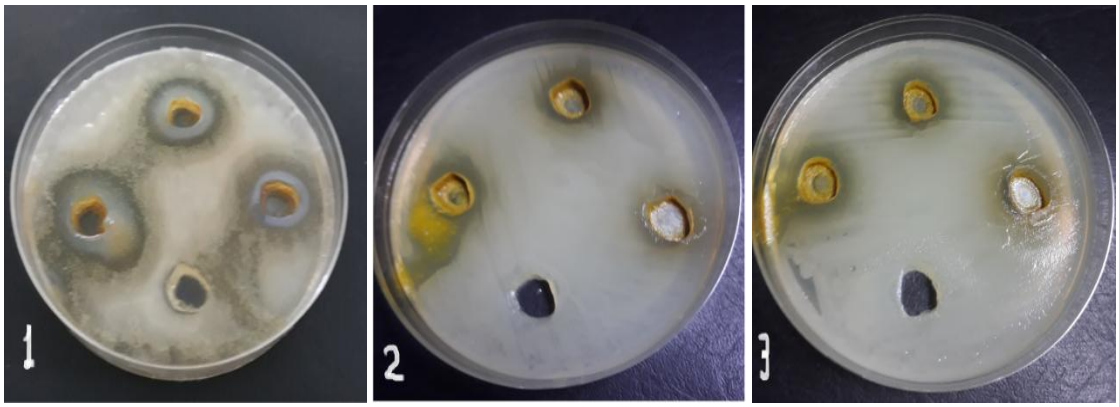

Figure 3: Effect of methanol extract against 1- S.aureus, 2- E. faecalis and 3-P.aeruginosa at 50,100 and 150 mg/ml concentrations, respectively (from right to left) compared with control.

Klebsiella sp. The organic extracts showed variable results towards the tested bacterial species, moreover, the chloroform extract was the most effective extract against four bacterial species *S. aureus, P. aeruginosa, Klebsiella sp.*

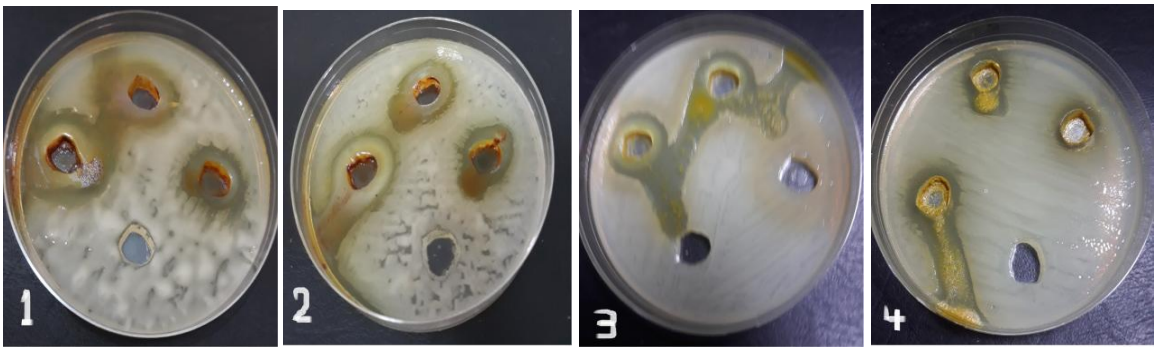

Figure 4: Effect of chloroform extract against 1- S.aureus, 2- P.aeruginosa, 3-Klebsiella sp. and 4- E.coli at 50,100 and 150 mg/ml concentrations, respectively (from right to left) compared with control.

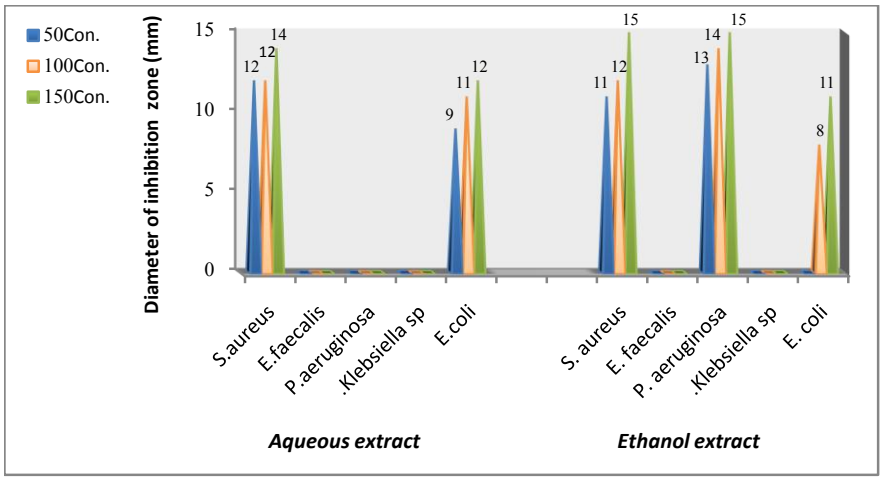

Figure 5: Effects of aqueous and ethanol extracts against different bacterial species

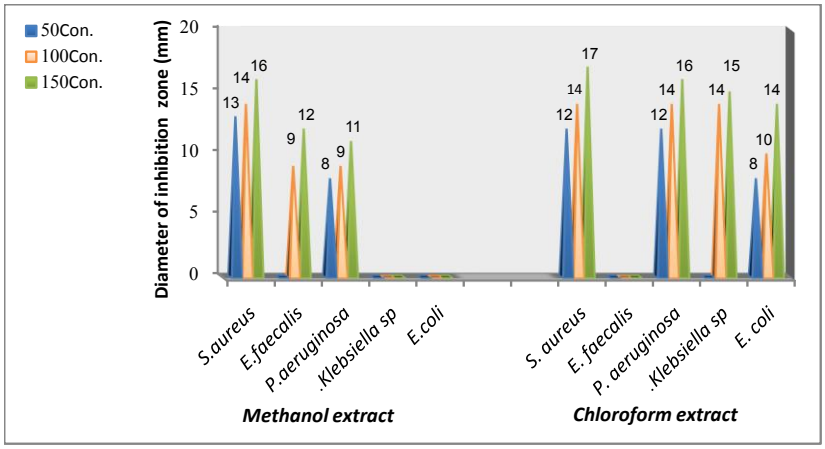

Figure 6: Effect of methanol and chloroform extracts against different bacterial species

and *E. coli* but show no activity against *E. faecalis.* While the other organic extracts exhibited an inhibitory effect against three bacterial species, as follows, the ethanol extract was effective against *S. aureus, P. aeruginosa,* and *E. coli*, but

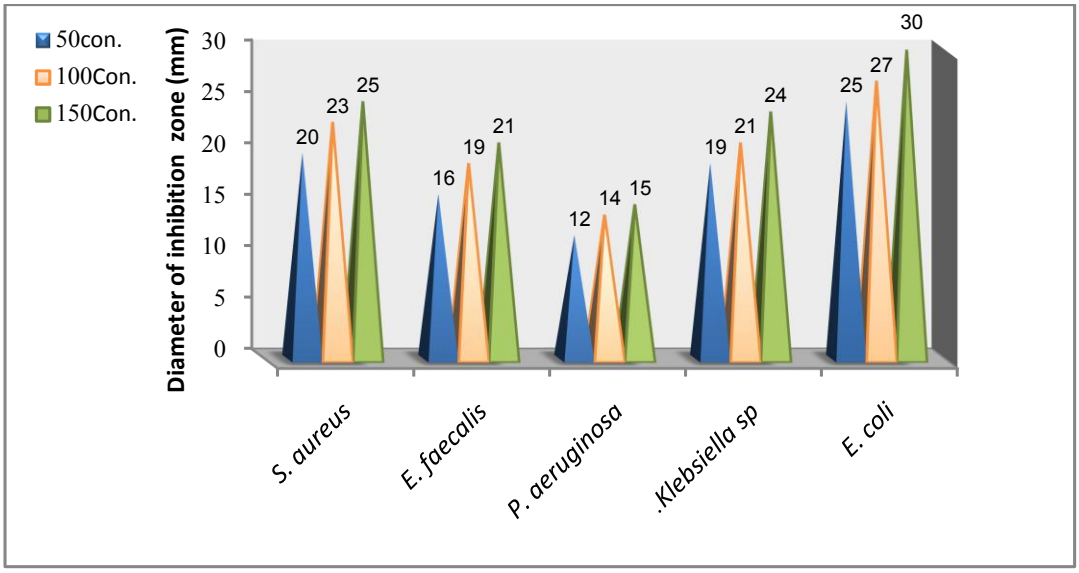

Figure 7: Effect of Gentamycin against different bacterial species

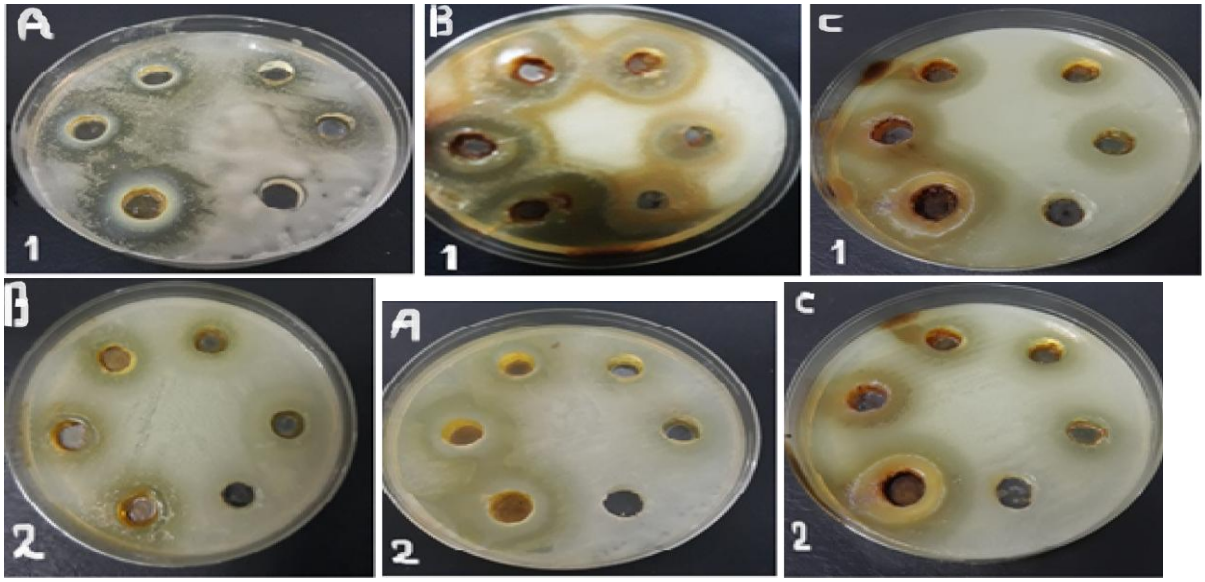

Figure 8: MIC's of the organic extracts: (A- ethanol, B- methanol and C- chloroform) against 1) *S. aureus* and 2) *P. aeruginosa* at 6.25, 12.5, 25, 50,100 and 200 mg/ml concentrations, respectively (from right to left).

show no effective against *E. faecalis* and *Klebsiella sp*. The methanol extract exhibited activity against *S. aureus, E. faecalis* and *P. aeruginosa* and show no activity against *E. coli* and *Klebsiella sp*. *Silene succulent* plant showed less antibacterial activity when compared with Gentamycin. The obtained results of the antibacterial activity of *S. succulent* leave extracts can suggest that *E. faecalis* and *Klebsiella sp.* were the most resistant species to most plant extracts, while *S. aureus* and *P. aeruginosa* were the most susceptible species to all the extracts. Especially the organic extracts were very effective at concentration of 150 mg/ml against *S. aureus* and *P. aeruginosa*, therefore, to determine the minimum inhibitory concentration (MIC) against these susceptible species, experiments on these organic extracts were conducted. The results of the MIC were recorded in (Table 2 and Figures 8 and 9). The inhibitory effects of all tested organic extracts started at 12.5 mg/ml with an inhibition zone diameter of 9, 10, and 11 mm against *S.aureus*. Whereas the inhibitory effect of methanol and chloroform extracts was 25 mg/ml with an inhibitory zone

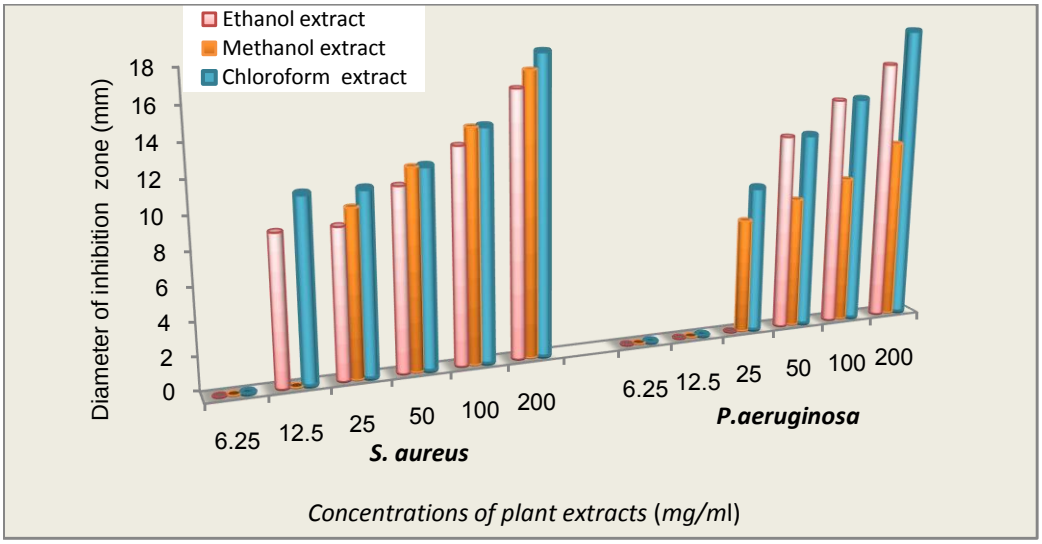

Figure 9: MIC of the effective plant extracts against *S. aureus* and *P. aeruginosa*.

diameter of 7 and 9 mm against *P.aeruginosa*, the inhibitory effect of ethanol extract was less effective and its MIC reached 50 mg/ml against the same bacteria with an inhibitory zone diameter of 12 mm.

DISSCUSION

The agar well diffusion method has been used in this research because it is more sensitive than the agar disc diffusion method (Valgas et al., 2007). In the present study, the obtained results from *Silene succulent* extras showed that *S. aureus* exhibited lower (MIC) than *P. aeruginosa*. These results are in accordance with the previous studies that reported that the minimum inhibitory concentrations of some plant extracts showed that *S. aureus* exhibited lower (MIC) than *P. aeruginosa* (Gonelimali et al., 2018; Mogana et al., 2020; Semeniuc et al., 2017; Sofy et al., 2017). Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganisms (Aqil, 2005) and many components of plant products have been shown to be specially targeted against resistant pathogenic bacteria (Nostro et al., 2006). The antimicrobial activity of plants is mostly due to the essential oils present in their composition. High phenolic compounds, flavonoids, aldehydes, ketones, saponins, and alcohols cause antimicrobial activity (Akgul, 1989; Sindhu and Manorama, 2012). It is known that these compounds including the Caryophyllaceae family. The flavonoids from plant extracts have been found to possess antimicrobial and antioxidants properties in various studies (Amarlal et al., 2009; Lin et al., 2008). Important characteristics responsible for the antimicrobial action of essential oils and extracts include hydrophobic components that allow the participation of lipids from the bacterial cell membrane, which disturbs cell structures and make them more permeable (Sikkema et al., 1994). Chemical compounds from essential oils and extracts also act on cytoplasmic membrane proteins (Knobloch et al., 1989).

CONCLUSION

It can be suggested that the degree of antimicrobial activity depends on the method of extraction, the type and amount of extracts, and the chemical properties of compounds. According to the results of this research and other studies that were performed on different species of *Silene*, it can be concluded that the antimicrobial potential of this plant is confirmed and its extractions are suitable to control pathogenic microorganisms and avoiding health hazards of chemically antimicrobial agent applications.

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