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Comparative study of antibacterial effect of ethanolic leaf extracts *Suaeda aegyptiaca* plant and some antibiotics in the growth of pathogenic bacteria

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ABSTRACT

The Antimicrobial properties of the ethanolic leaf extract of the *Suaeda aegyptiaca* plan, on four bacterial isolates (*Stapshylococcus aureus, S.epidermidis, P.aeruginosa,and Escherichia coli*),were investigated . The antimicrobial activity of the plant extract was determined by the Well diffusion method. The ethanolic leaf extract of all the plants inhibited the test organisms. The spectrum of antimicrobial activity was recorded against *S. aureus*(26.66-14.66 mm),*S.epidermidis*(22-15.22 mm), *P. aeruginosa*(20.33-13.33 mm), *and E.coli* (21.66-18.55 mm) at (10-2.5%) con.

The studying extract exhibited antimicrobial properties compared favorably with some standard antibiotics .The maximum zone of inhibition of antibiotics was measured against *S.aureus*was (E) (16mm), on the other hand, (ST and AM) had no effect on its. *S.epidermidis* was highly sensitive to (NOR) was 38mm and resistant to (AM and L). *E.coli* and *P.aeruginosa* was highly sensitive to (AM),while it is resistant to (CAZ,PY and CXM).As well as AMC and CD had no effect on *P.aerug - inosa*.Compare with The screening results of the medicinal plant extract in the present study confirmed a source of antimicrobial agents by the highest sensitivity was recorded.

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INTRODUCTION

Many of antibiotics have failed to discourage the growth of many bacteria that have genetic ability to transmit and acquire resistance to drugs, In addition to the side effect of these antibiotic which can harm vital organs like liver, kidneys, the pancreas and spleen as well as their impact on the immune system (Cohen, M.L., 1992 ; Driscoll, J.A., S.L. Brody and M.H. Kollef, 2007). Because of the side effects and bacteria resistance against the antibiotics, the scientists developed new drugs from natural sources such as plants, which have been extensively used as alternative treatment for disease as antibacterial, antifungal, antioxidants and anticancer due to that most of these plants contain many active compounds such as flavonoids, tannis, saponins, alkaloids, terpenes, heavy metals.The known success of traditional medicine has guided the search for new chemotherapeutic alternatives to eliminate the infections caused by drug-resistant microbes and to reduce the harm caused by antibiotic (Bocanegra-Garcia, V. et al., 2009). However, herbal extracts have found it often to antimicrobial growth enhancers in animal feed due to the residual effects that leave for restricted use. These cases as instances of anti-bacterial, anti-oxidant, anti-cancer, anti-fungal, relaxing, pesticides and insecticides, as well as growth enhancers are introduced (Manoj *et al.,*2010).*Suaeda aegyptiaca* is a species of plant in the family Amaranthaceae, that grow naturally in salt-affected areas, in folk medicine, the herb *S. aegyptiaca* has been used for stomach pain and for wound and skin infection (Ghazanfar 1994). The name Suaeda come from Arabic word —Suwaid meaning —Black because the plant was turning darkeror blackish when dried (Torkelson 1996).The study was aimed at determining the in vitro antibacterial activity of present ethanolic extract by investigating its effects on inhibition of biological activity of bacteria with the view to finding alternative means of treating infections caused by them .

MATERIALS AND METHODS:

The *Suaeda aegyptiaca* leaves were purchased from different areas of Thi-Qar . The plants were dried, powdered and stored in a sterile container until use.

Preparation of crude extracts:

The methods of Akujobi*et al*., (2004) and *Esimone et al.*, (1998) were adopted for the study. Powdered sample (20 g) was extracted in a soxhlet apparatus with 200 ml of solvent at the room temperature. The samples were stored at 40C until use.Stock solution of 20 mg /ml was prepared. Stock solutions were prepared one day in advance. Multiple aliquots of each sample were stored for initial tests and retests, if necessary. Stock solutions were filtered sterilised. On the day of assay, thaw an aliquot of frozen stock solution at room temperature. Prepared 100 µg/ml concentration of the extract by serial dilution of stock solution , The crude extracts obtained were diluted to obtain (10% ,5% and 2.5%) concentrations .

Test microorganisms and their sources:

The isolates *Staphylococcus aureus, Staphylococcus epidermides, Escherichia coli and Pseudomonas aeruginosa* were obtained

well aseptically and were incubated at 37˚C for 24 hours. The zone of inhibition was measured. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. DIZ was expressed in millimeters. All tests were performed in triplicates by methods of Cheesbrough (2001).

Sensitivity test of standard antibiotics

Sensitivity of antibiotics against test strains was assessed by agar disc diffusion method (Baur et al., 1966). Seven stander antibiotics tested against Gram negative bacteria are Amikacin (AK,30mcg), Oxytetracycline (T, 30mcg) , Amoxicillin/clavulanic acid (AMC, 30 mcg, 20/10 mcg), Ceftazidime (CAZ,30mcg), Cefdinir (CD,5mcg), Carbeniciln (PY,25mcg) and Cefuroxime (CXM, 30mcg). Norfloxacin (NOR, 10mcg), Sulfisoxazole (ST, 300), Erythromycin (E, 15mcg), Ampicilin (AM,25) and Lincomycin (L 2mcg) were tested against Gram positive bacteria. Sensitivity was predicted with degree of clear zone surrounding the disc after 24 h in mm (Barry *et al.,* 1979).

Statistical Evaluation:

American statistical program (SPSSl8) was used to analyzed data by using simple statistics of ANOVA. The mean was separated using Least Significant Difference (LSD). (> 0.5)

from Thi-Qar University College of Science, Department of Biology, Identification of bacterial species was confirmed using API Staph. and API Enterobacteracea (Collee *et al.,* 1996),The bacteria were isolated from clinical specimens. The pure cultures subcultured on Nutrient agar slants. They were stored at 40C until required for the study.

Antibacterial Assay

In vitro antibacterial of crude ethanolic extract by the well diffusion method. This method was detected according to (NCCLS, 2002). methanolic extracts of Suaedaaegyptiaca leaves, Zingiber officinale rizomes, Curcuma longa rizomes and Cinnamomum zeylanicum bark screened for antimicrobial activity by this method.

Kirby bauer Agar Well Diffusion method was used to study the effect of various bark extracts on the selected bacterial strains. The sterilized nutrient agar medium was aseptically poured (20ml) into the sterile petri-plates and allowed to solidify. The bacterial broth cultures were separately swabbed on petri-plate using a sterile bud. Wells (5 mm in diameter) were made from the agar with a sterile borer. The organic extracts of plants (30 µl) were added to each

RESULTS AND DISCUSSION

I. Preparation of extracts

The results of the ethanol extraction of the studying plant by theSoxhlet apparatus can be seen in

Scientific name	Common name	Extract Color in Soxhlet apparatus (Dark green)	Part used
Suaedaaegyptiaca	Suaeda	 	Leaves

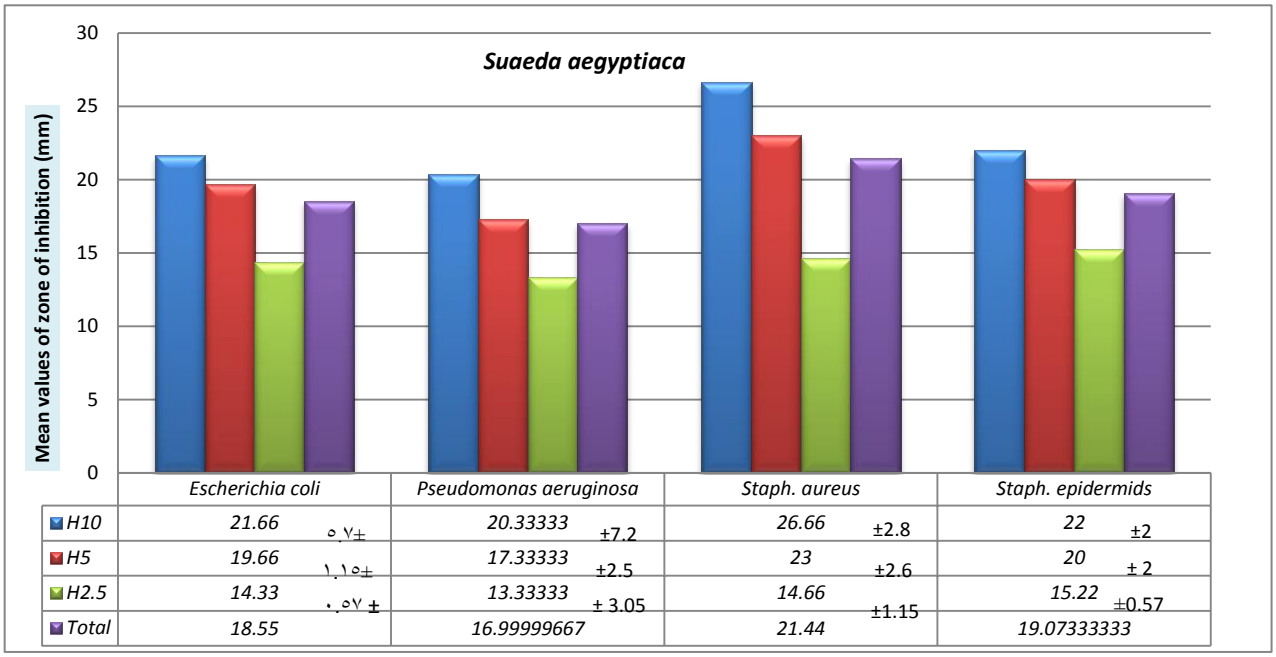
table (1).

Table1. Extraction Results from the studying plants Powder

inhibition was produced by the 2.5% concentration on *P.aeruginosa*which gave a zone of growth inhibition measuring 13.33 mm.Where the results shows the spectrum of antimicrobial activity was recorded against *S. aureus*(26.6614.66mm),while*S.epidermidis*(22-15.22mm),*P.aeruginosa*(20.33-13.33mm),*and E.coli* (21.66-18.55 mm) at (10-2.5%) con.Gram-negative *P. aeruginosa* is known to have a high level of intrinsic resistance to virtually almost all known antimicrobials and antibiotics, due to a very restricted outer membrane barrier, highly resistant even to synthetic drugs (Mann and Markham, 2000).

The Antimicrobial activity of studying plants Againsts Bacteria:

Based on the results of the antimicrobial assay using the agar diffusion method (well were made in medium agar, which were filled with sample extracts) ethanol extracts of *studying plant* has great antimicrobial inhibition zones against *against E. coli, P.aeruginosa,S. aureus and S.epidermidis*.**Fig.1** shows the results of the antimicrobial screening of the crude ethanolic extract of *S. aegyptiaca.*The largest zone of inhibition was produced by the 10% con. On *Staph aureus* with a zone diameter of 26.66 mm. The lowest zone of



	Escherichia coli	Pseudomonas aeruginosa	Staph. aureus	Staph. epidermids
H10	21.66 ٥.٧±	20.33333 ±7.2	26.66 ±2.8	22 ±2
H5	19.66 ١.١٥±	17.33333 ±2.5	23 ±2.6	20 ± 2
H2.5	14.33 ٠.٥٧ ±	13.33333 ± 3.05	14.66 ±1.15	15.22 ±0.57
Total	18.55	16.99999667	21.44	19.07333333

LSD.(0.05) between cons. **{ E. coli = 7.33 ;** *P.aeruginosa* **=N.S ;** *Staph aureus* **= 8.33 and** *S.epidermidis***= 4.66 }**

activities, etc. The leaf of *S. aegyptiaca*has been used as a medicine for hepatitis and antiviral activity (Alhdad*et al.,* 2013 and Reda*et al.,* 2004).

Fig. 2 Shows antibacterial activity of *aegyptiaca* ethanolic extracts of *the Suaeda* extractsagainst the gram positive and gram negative human pathogenic bacteria. All the extracts studied in the present investigation exhibited varying degree of inhibitory effect against all the tested human pathogenic bacteria.

Fig. 1 shows the results of the crude ethanolic extract of *Suaeda aegyptiaca* against four This observation due to pathogenic bacteria. active chemical compounds in this extract which agrees with the previous studies shown that the bioactive molecules of the leaf of *Suaedamomocia*of the various extracts showed the presence of alkaloids, steroids, coumarins, catachins, tannins, phenols, flavonoids, saponins, glycosides and xanthoprotein. Traditionally, the leaf from *Suaedamonoica*is known to use as a medicine for hepatitis and scientifically it is reported to be used as ointment for wounds and possess antiviral activity, because of the presence of triterpenoids and sterols (Ravikumar et al.,2010). Evaluation of the vitamin B group *aegyptiaca*can be composition of various *Suaeda* used to achieve the levels and importance of ions in different weather conditions and also obtain the organs of plant that can be used to prepare an extract of important and useful ions for humans. Leaves of the plant have been traditionally used as a medicine for hepatitis and it has been reported antibacterial, antioxidant

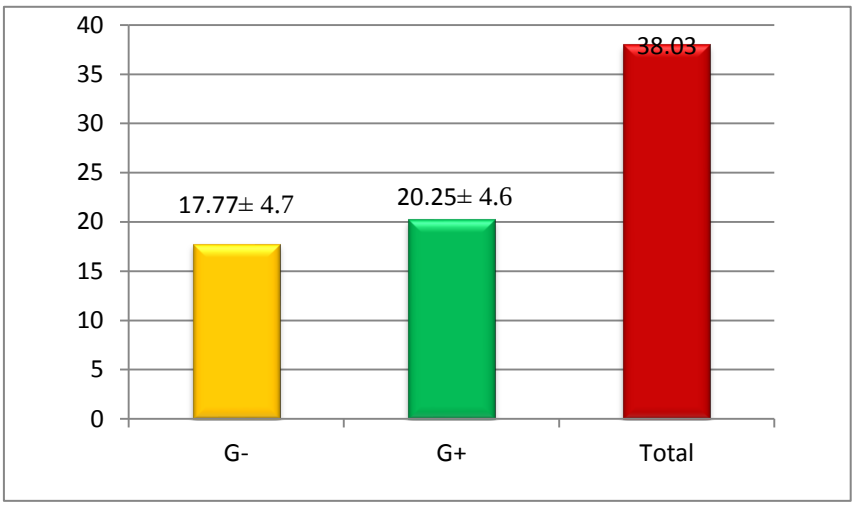


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Fig. 2: The results of the crude ethanolic extract of *Suaeda aegyptiaca* against the G+ and G- bacteria.

bonding to membrane proteins and destructing the membranes, electron transport systems and cell wall (Wahle*et al*., 2010).

Antimicrobial activity of antibiotics and Medicinal plants ethanolic extracts

Table **1** shows the effect of antibiotics against gram negative bacteria.Sensitivity test revealed that the *E.coli* was highly sensitive to Amikacin and Ox tetracycline were (24 and 20 mm respectively).The results also revealed that Amoxicillin/clavulanic acid had moderate effect as 13 mm (zone of inhibition) while Cefdinir had lowest effect as 8 mm . On the other hand *E.coli* was resistant against Ceftazidime,Carbeniciln and Cefuroxime. Compare with the screening results of the medicinal plants extracts in the present study confirmed a source of antimicrobial agents by the highest sensitivity The zone of inhibition 3 was recorded (fig). was recorded highly sensitive to Amikacin and Oxytetracycline were (28 and 20mm

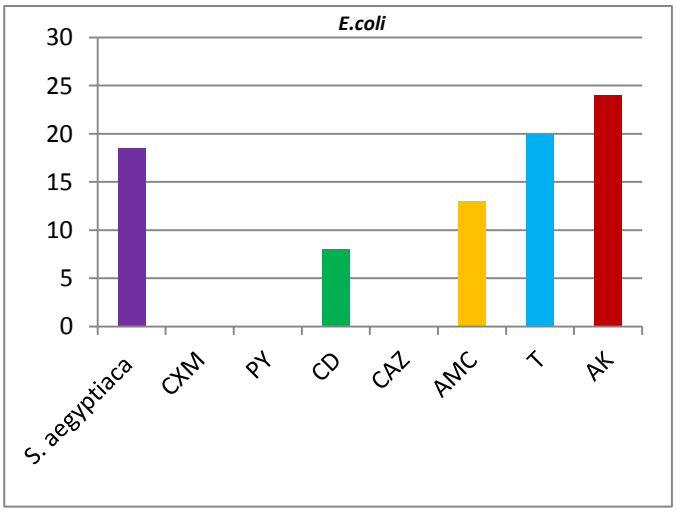
The results shows the higher inhibition zone of all extracts for gram positive which ranged between 20.94 mm to 18.83 mm compare with gram negative bacteriawhich ranged between 17.55 mm to 14.38 mm**.** AlsoIt can be seen antibacterial activity by *Suaeda aegyptiaca* extracts 38.03 mm.Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antibacteria, antioxidant and antitumor activity.The results indicate the activity of present extracts was more effective against Gram-positive than Gram-negative bacteria,this fact is in agreement with previous reports (Kelmanson et al., 2000, Mesika and Atolsyane 2002) .The higher resistance of Gram-negative bacteria against plant extracts is credited to the presence of outer membrane lipopolysaccharides, also these observations are likely to be the consequences of the differences in cell wall structure between Gram-positive and Gram-negative bacteria. Phenolic compounds are capable of further cellular destruction and inhibition by establishing the hydrophobic and hydrogen

Medicinal plants ethanolic extracts against *P. aeruginosa* (fig4).

respectively) against *P.aeruginosa* .whileAmoxicillin/clavulanic acid , Cefdinir , Ceftazidime ,Carbeniciln and Cefuroxime had no effect of its(table 2),Compare with the highest sensitivity was recorded by

Table 1: The effect of antibiotics against gram negative bacteria

Antibiotics		Conc. Of antibiotics [µg]	Mean values of zone of inhibition (mm)	
			Escherichia coli	*Pseudomonas aeruginosa*
Amikacin	AK	[30]	24	28
Oxytetracycline	T	[30]	20	20
Amoxicillin/clavulanic acid	AMC	[30] 20/10	13	R
Ceftazidime	CAZ	[30]	R	R
Cefdinir	CD	[5]	8	R
Carbeniciln	PY	[25]	R	R
Cefuroxime	CXM	[30]	R	R



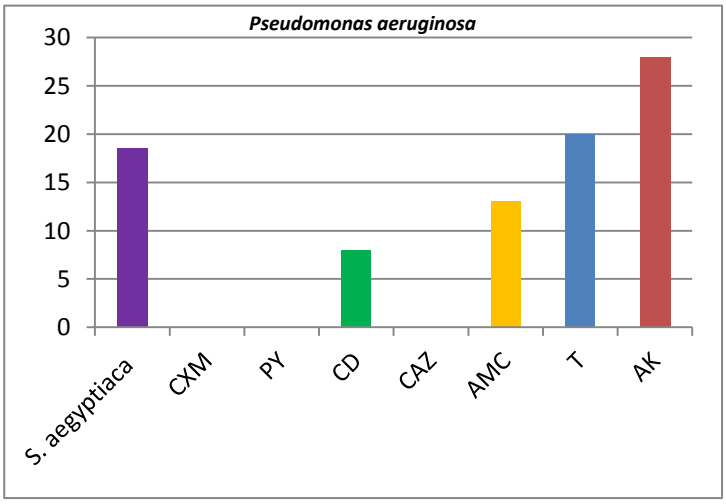


Figure 3 ,4 Effect of ethanol extracts of *S.aegyptiaca*and some antibiotics and ethanolic plant

S. epidermidis was highly sensitive to Norfloxacin where the zone of inhibition recorded as 38 mm,while. Sulfisoxazole showed lowest effect on the growth of *S. epidermidis* as 8 mm. On the other hand Erythromycin, Ampicillin, and Lincomycin had no effect on it.Compare with the highest sensitivity was recorded by Medicinal plants ethanolic extracts against*S. aureus*and *S. epidermidis*(fig 6).

Table 2 shows the effect of antibiotics against gram positive bacteria. The zone of inhibition of antibiotics was measured against *S. aureus* were Erythromycin andNorfloxacin recorded the maximum zone of inhibition was (16 and 15 mm) respectively (fig.5 and table 1), while low susceptibility was measured byLincomycin (9mm). On the other hand,Sulfisoxazole andAmpicilinhad no effect on *S. aureus*.

Table 3:Effect of some antibiotics and ethanolic plant extracts on the G+ bacteria

Antibiotics		Conc. Of Antibiotics [µg]	Mean values of zone of inhibition (mm)	
			Staphylococcus aurues	***Staphylococcus epidermidis***
Norfloxacin	NOR	[10]	15	**38**
Sulfisoxazole	ST	[300]	R	**8**
Erythromycin	E	[15]	16	**R**
Ampicilin	AM	[25]	R	**R**
Lincomycin	L	[2]	9	**R**

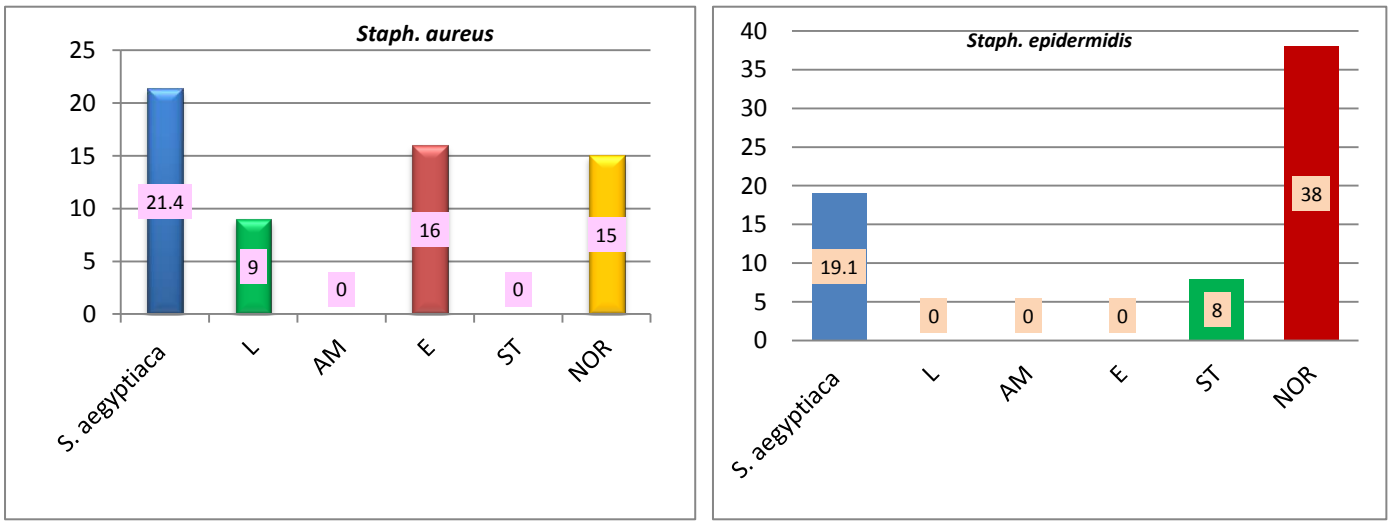
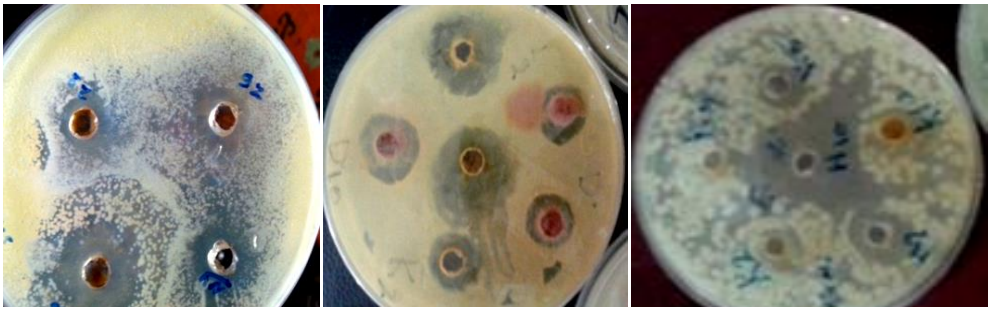
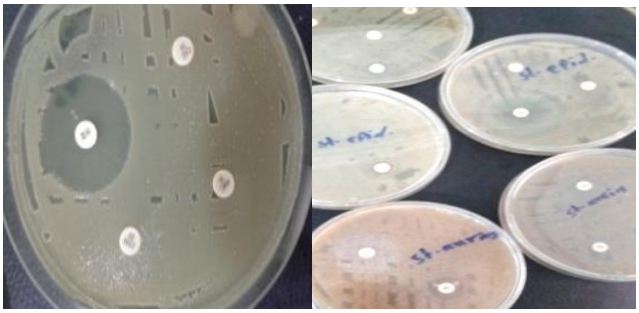


Figure (5,6): Effect of ethanol extracts of *S.aegyptiaca*and some antibiotics and ethanolic plant

extracts on the G+ bacte



PICs. : shows Inhibition zone produced by present plant extract on tested bacteria.



PICs. : shows Inhibition zone produced by standard antibiotics on tested bacteria.

penetrating ability of extracts in to bacterial cells (Walsh *et al.*, 2003 and Oussalah*et al.,* 2006). Further studies are needed to find out the active compounds of these plants.

Conclusion

We concluded that, it is possible to find better therapies for many infectious diseases from the plant extracts.Pharmacological test is necessary to isolate and characterize their active compounds. It is possible to find better therapies for many infectious diseases from the plant extracts.

The findings of this study showed that present extracts had inhibited both Gram-positive bacteria and Gram-negative bacteria indicating broad spectrum inhibitory effect. Gram positive bacteria were more susceptible than Gram-negative bacteria by the action of extracts, demonstrating antibacterial effect which was comparable with that of the standard drugs.

Many studies reported the incapability of herbal antimicrobial agents to inhibit growth of Gram-negative bacteria due to the presence of complex cell wall structure which decreases the penetration of bacterial cells by herbal extracts. But in the present study extracts shows active zone inhibition agaict the growth of many bacteria proving

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