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IN VITRO EVALUATION ANTIMICROBIAL ACTIVITIES OF *CINNAMOMUM ZEYLANICUM* AND COMPARED WITH SOME ANTIBIOTICS

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ABSTRACT
The Antimicrobial properties of the ethanolic extract of *Cinnamomum zeylanicum* bark were assayed for the in vitro antimicrobial activity against gram-positive standard bacteria represented by *Staphylococus aureus* and *Staphylococcus epidermides* and gram-negative standard bacteria represented by *Escherichia coli* and *Pseudomonas aeruginosa* using holeplate diffusion method. All the extracts studied in the present investigation exhibited varying degree of inhibitory effect against all the tested human pathogenic bacteria .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 bacteria which ranged between 17.55 mm to 14.38 mm. Also It can be seen antibacterial activity of *Cinnamomum zeylanicum* is 33.22 mm. Compare with The screening results of the Sensitivity test which revealed that the *E.coli* was highly sensitive to Amikacin and Oxytetracycline 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. The zone of inhibition was recorded highly sensitive to Amikacin and Oxytetracycline were (28 and 20 mm respectively) against *P. aeruginosa*.while Amoxicillin/ clavulanic acid, Cefdinir, Ceftazidime, Carbeniciln and Cefuroxime had no effect of its. The zone of inhibition of antibiotics was measured against *S. aureus* were Erythromycin and Norfloxacin recorded the maximum zone of inhibition was (16 and 15 mm) respectively, while low susceptibility was measured by Lincomycin (9mm), On the other hand Sulfisoxazole and Ampicilin had no effect on *S. aureus*. *S. epidermidi*s was highly sensitive to Norfloxacin where the zone of inhibition recorded as 38mm, while. Sulfisoxazole showed lowest effect on the growth of *S. epidermidi*s as 8 mm. On the other hand Erythromycin, Ampicillin, and Lincomycin had no effect on it. Compare with The screening results of the medicinal plants extracts in the present study confirmed a source of antimicrobial agents by the highest sensitivity was recorded of its.

KEYWORDS: *Cinnamomum zeylanicum, Staphylococus aureus, Staphylococcus epidermides.*

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 scientist 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.

Medicinal Plants are rich source of natural products used for centuries to cure various diseases where its have a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinines. The World Health Organization (WHO) has estimated that approximately 80% of the global population relies on traditional herbal medicines as part of standard healthcare (Foster et al., 2005). Many drugs presently prescribed by physicians are either directly isolated from plants or are artificially modified versions of natural products. In Western countries, approximately 25% of the drugs used are of natural plant origin (Payne et al., 1991).

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)

Cinnamomum zeylanicum tree belongs to the family, Lauraceae most noted for its bark, which provides the world with the commonly known culinary spice, cinnamon. Cinnamon has medicinal property and has been used to treat gastrointestinal complaints and other ailments (Cao and Anderson, 2011). Cinnamon possesses antiallergenic, anti-inflammatory, anti-ulcerogenic, anti-pyretic, antioxidant, anaesthetic activities (.Lin et al., 2003). Antioxidant studies with *Cinnamomum zeylanicum* bark showed better free radical scavenging capacity against a battery of free radicals (Varalakshmi,2012) . Cinnamomum bark oil possesses the aroma of the spice and a sweet pungent taste. It is employed mainly in the flavouring industry where it is used in meat and fast food seasonings, sauces, pickles etc., and also in pharmaceutical preparations. Cinnamaldehyde (75%) and Camphor (56%) are the major constituents of Cinnamomum in stem bark and roots. Cinnamomum leaf oil is a valued source of eugenol (90%) with phenols 78-95% and aldehyde 5% Apoptosis is the mode of cell death induced by stimuli such as drugs, stress, radiation etc., The study was aimed at determining the in vitro antibacterial activity of present ethanolic extracts 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 *Cinnamomum zeylanicum* bark was purchased from local market of Thi-Qar . The plants was 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 room temperature. The samples were stored at 40C until use. Stock solution of 20 mg /ml were 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 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 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 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 posative bacteria. Sensitivity was predicted with degree of clear zone surrounding the disc after 24 h in mm (Barry *et al.*, 1979).

Statistical Evaluation

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of duplicates ±SD of two triplicates.

RESULTS AND DISCUSSION

I. Preparation of extracts

The results of the ethanol extraction of the studying plants by the Soxhlet apparatus are quite different in color and weight from each other this is likely due to

differences in the nature of the parts of these plants and the chemical components of its in the solvents. Color and Weight of the studying extracts can be seen in Table 1.

Table 1: Extraction Results from the studying plants Powder.

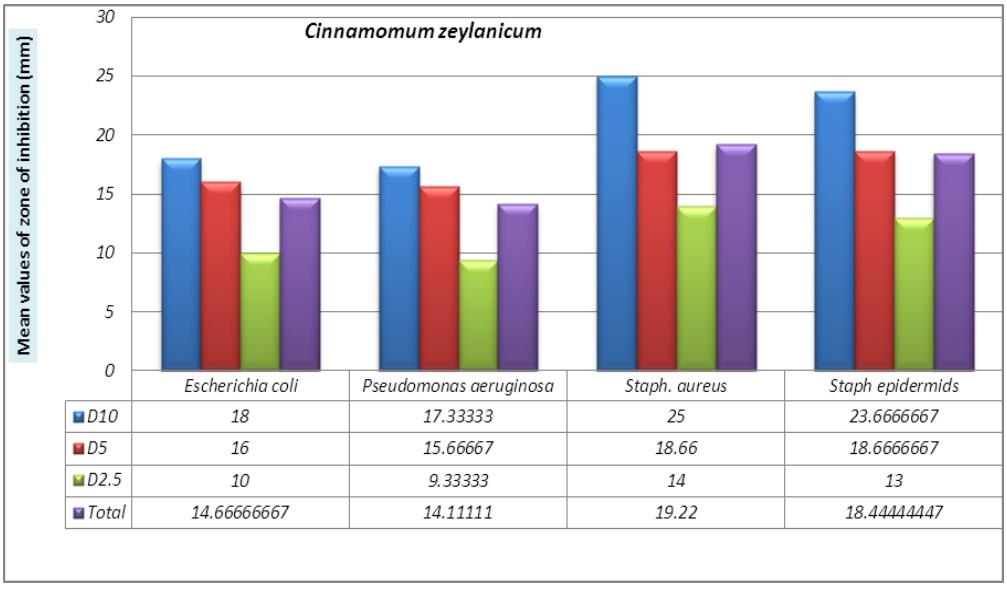
Scientific name	Common name	Part used	Extract Color (pic. 1,2,3and 4)	Extract Weight (gram)
Cinnamomum zeylanicum	Cinnamon	Bark	Dark brown	

(Curcuma longa)) ***((Suaeda aegyptiaca)*** ***(Cinnamomum zeylanicum)*** ***(Zingiber officinale)***

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 plants* has great antimicrobial inhibition zones against *against E. coli, P.aeruginosa, S. aureus and S.epidermidis*.

Fig. 3, 4 shows the results of the antimicrobial screening of the crude ethanolic extract of *Cinnamomum zeylanicum* and *Suaeda aegyptiaca* respectively . The largest zone of inhibition was produced by the 10% con. on *Staph aureus* with a zone diameter of 25 mm and 20.33 mm respectively. The lowest zone of inhibition was produced by the 2.5% concentration of *Cinnamomum zeylanicum* and *Suaeda aegyptiaca* on *P.aeruginosa* which gave a zone of growth inhibition measuring 9.33 mm and 13.33 respectively.



	Escherichia coli	Pseudomonas aeruginosa	Staph. aureus	Staph epidermids
D10	18	17.33333	25	23.6666667
D5	16	15.66667	18.66	18.6666667
D2.5	10	9.33333	14	13
Total	14.66666667	14.11111	19.22	18.44444447

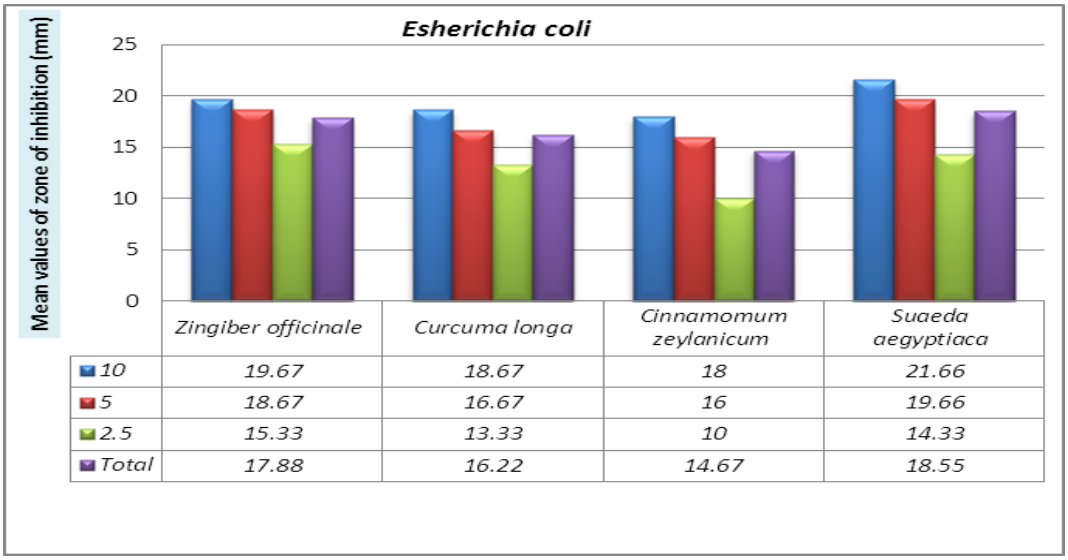
Results agree with research investigation by Mandal *et al. (*2011) reported that Cinnamon bark is rich in cinnamaldehyde which has been proven to be active against many pathogenic gram positive and gram negative bacteria.

The antimicrobial character of cinnamon is mostly due to two major phytochemicals cinnamic aldehyde from the bark (65 to 75%) (Lopez-Malo *et al*., 2000) and eugenol from the leaf (80%).

Cinnamaldehyde acts on bacteria by inhibiting the bio-synthetic enzymes (Walsh *et al.*, 2003). It can also have an effect on a specific enzyme of *H. pylori* (urease). According to Prabuseenivasan et al. (2006), the inhibitory effect of EO of *C. zeylanicum* may be related to the reduction of intracellular pH of this bacterium (Oussalah et al., 2006). Some molecules of oil (cinnamaldehyde and cinnamyl) bind to membrane proteins and inhibit peptidoglycan synthesis, the essential component of the bacterial cell wall, thereby increasing their antibacterial effect.

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).

Fig. 5 Illustrates the effect of the *Zingiber officinale*, *Curcuma longa*, *Cinnamomum zeylanicum* and *Suaeda aegyptiaca* extracts on *Esherichia coli*.



	Zingiber officinale	Curcuma longa	Cinnamomum zeylanicum	Suaeda aegyptiaca
10	19.67	18.67	18	21.66
5	18.67	16.67	16	19.66
2.5	15.33	13.33	10	14.33
Total	17.88	16.22	14.67	18.55

The higher mean zone of inhibition was found to be 21.66 mm at 10% con. of *Suaeda aegyptiaca* extract. While the other zones of inhibition are 19.66-15.33mm, 18.66-13.33mm and 18-10mm for *Zingiber officinale*, *Curcuma longa*, *Cinnamomum zeylanicum* respectively at 10%, 5%, 2.5% con.

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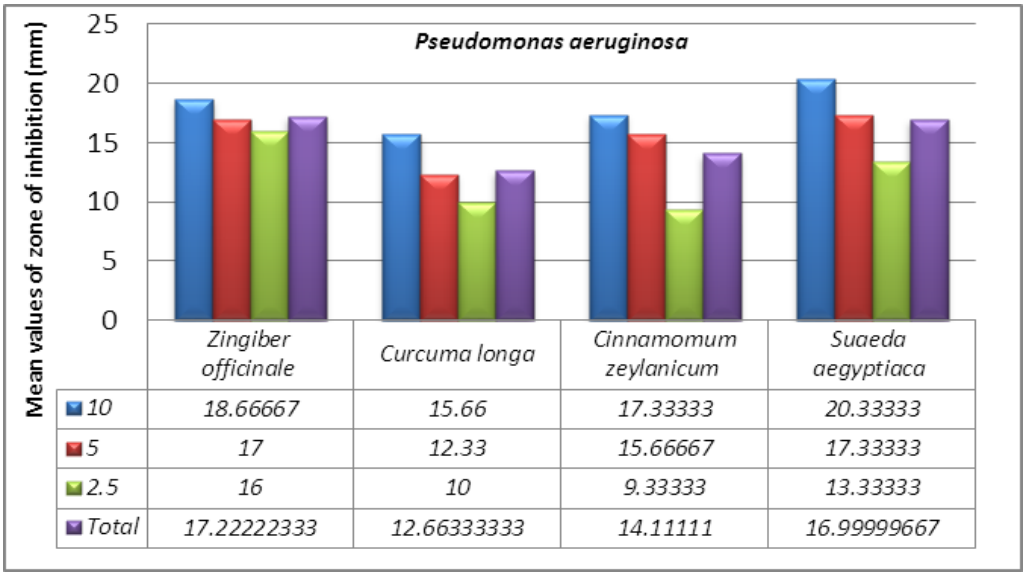
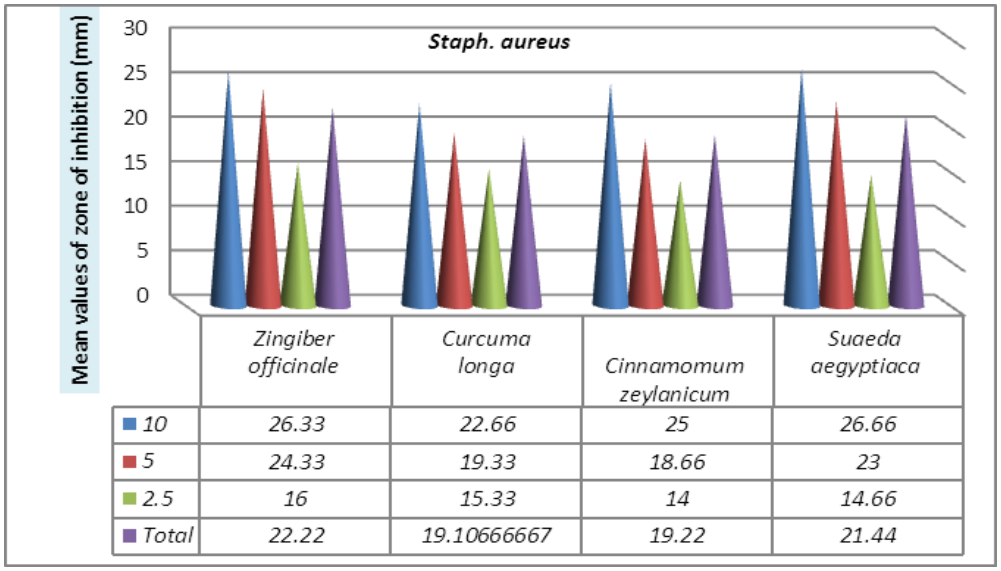


	Zingiber officinale	Curcuma longa	Cinnamomum zeylanicum	Suaeda aegyptiaca
10	18.66667	15.66	17.33333	20.33333
5	17	12.33	15.66667	17.33333
2.5	16	10	9.33333	13.33333
Total	17.22222333	12.66333333	14.11111	16.99999667

(Fig. 6): Illustrates the effect of the *Zingiber officinale*, *Curcuma longa*, *Cinnamomum zeylanicum* and *Suaeda aegyptiaca* extracts on *P. aeruginosa*.

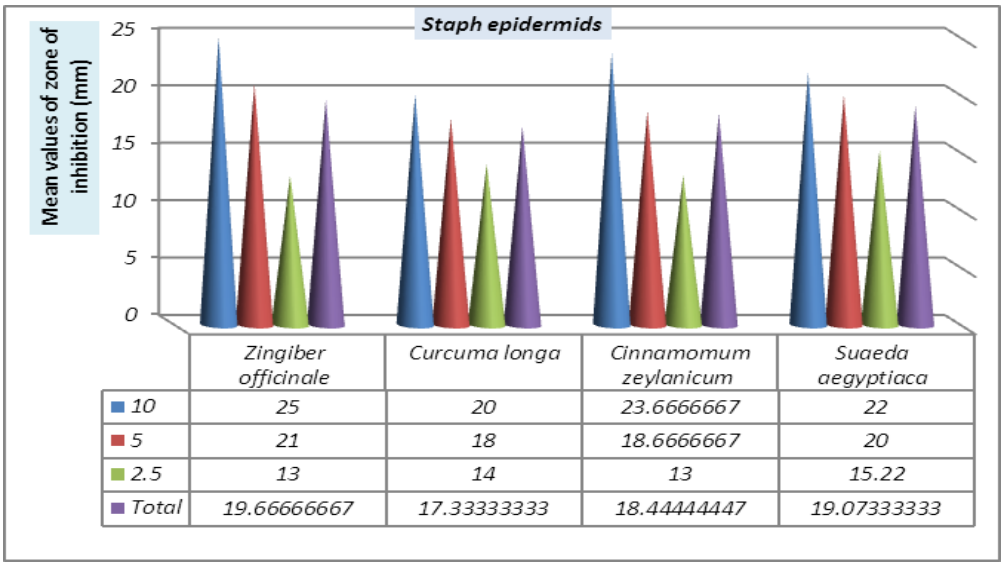
The higher mean zone of inhibition was found to be 20.33 mm at 10% con. of *Suaeda aegyptiaca* extract. While the other zones of inhibition are 18.66- 16 mm, 15.66-10mm and 17.33-9.33mm for *Zingiber officinale*, *Curcuma longa* ,*Cinnamomum zeylanicum* respectively at 10% ,5% , 2.5% con.



	Zingiber officinale	Curcuma longa	Cinnamomum zeylanicum	Suaeda aegyptiaca
10	26.33	22.66	25	26.66
5	24.33	19.33	18.66	23
2.5	16	15.33	14	14.66
Total	22.22	19.10666667	19.22	21.44

(Fig. 7): Illustrates the effect of the *Zingiber officinale*, *Curcuma longa*, *Cinnamomum zeylanicum* and *Suaeda aegyptiaca* extracts on *Staph aureus*.

The higher mean zone of inhibition was found to be 26.66 mm at 10% con. of *Suaeda aegyptiaca* extract. While the other zones of inhibition are 26.33-16 mm, 22.66-15.33 mm and 25-14 mm for *Zingiber officinale*, *Curcuma longa*, *Cinnamomum zeylanicum* respectively at 10%, 5%, 2.5% con.



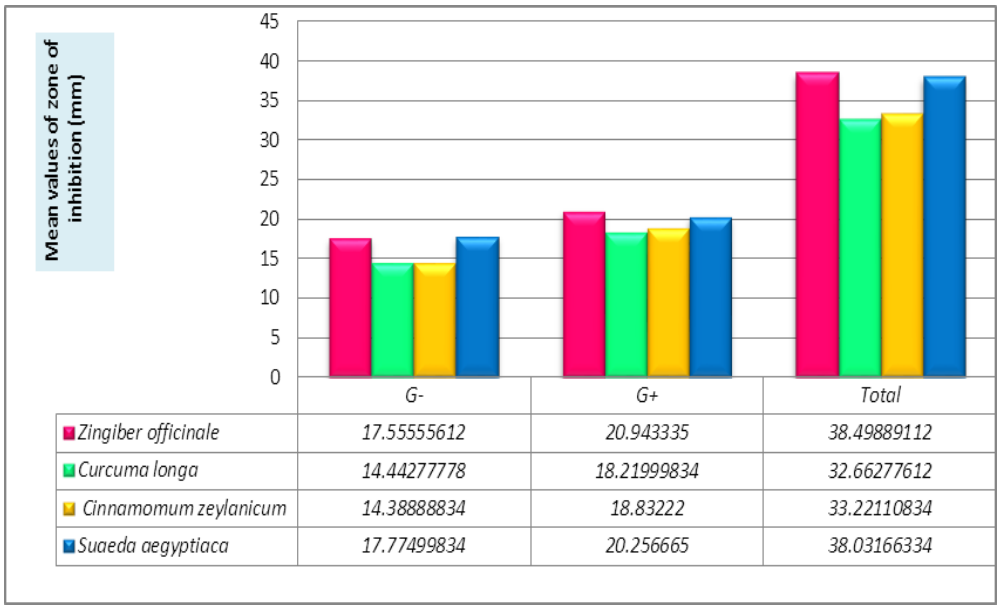
	Zingiber officinale	Curcuma longa	Cinnamomum zeylanicum	Suaeda aegyptiaca
10	25	20	23.6666667	22
5	21	18	18.6666667	20
2.5	13	14	13	15.22
Total	19.66666667	17.33333333	18.44444447	19.07333333

(Fig. 8): Illustrates the effect of the *Zingiber officinale*, *Curcuma longa*, *Cinnamomum zeylanicum* and *Suaeda aegyptiaca* extracts on *Staph epidermidis.*

The higher mean zone of inhibition was found to be 25 mm at 10% con. of *Zingiber officinale* extract. While the other zones of inhibition are 20-14 mm, 23.66-13 mm and 22-15.22 mm for *Curcuma longa* ,*Cinnamomum zeylanicum* and *Suaeda aegyptiaca* respectively at 10%, 5%, 2.5% con.

(Fig.7): Show antibacterial activity of ethanolic extracts of the *Zingiber officinale, Curcuma longa, Cinnamomum zeylanicum* and *Suaeda aegyptiaca* extracts against 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.



	G-	G+	Total
Zingiber officinale	17.55555612	20.943335	38.49889112
Curcuma longa	14.44277778	18.21999834	32.66277612
Cinnamomum zeylanicum	14.38888834	18.83222	33.22110834
Suaeda aegyptiaca	17.77499834	20.256665	38.03166334

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 bacteria which ranged between 17.55 mm to 14.38 mm**.** Also It can be seen antibacterial activity of *Zingiber officinale extracts* give higher inhibition zones *38.49* mm against *all* human pathogenic bacteria, followed by *Suaeda aegyptiaca* extracts 38.03 mm while the inhibition zones of *Curcuma longa and Cinnamomum zeylanicum* are 32.66mm and 33.22 mm respectively.

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 antibacterial, antioxidant and antitumor activity.

Alkaloids are known to exhibit emetic amoebicides, expectorant, anesthetics, antipyretics, analgesics, antileminthic and can be used for the treatment of stomach Certain plant phenols can be effective inhibitors of chemical mutagens, *in vitro*, and/or carcinogenesis in vivo (Singh et al., 1998).

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Tannins are plant polyphenolic compounds that are contained in large quantities in food and beverages (tea, red wine, nuts, etc.) consumed by humans daily. It has been shown that various tannins exert broad cancer chemoprotective activity in a number of animal models. These phenolic compounds are capable of further cellular destruction and inhibition by establishing the hydrophobic and hydrogen 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

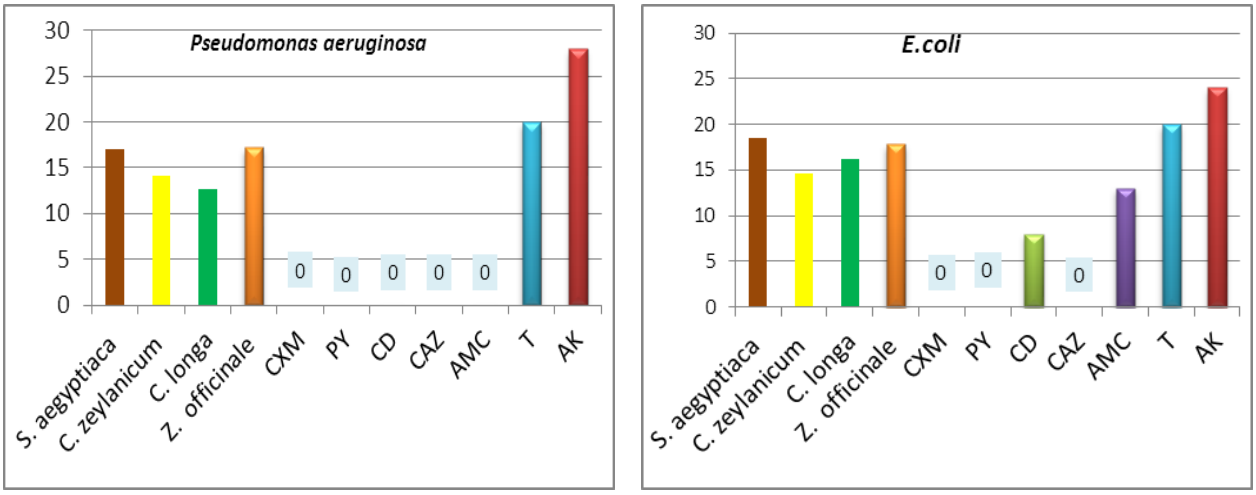
(Table 1): Show the effect of antibiotics against gram negative bacteria.

Sensitivity test revealed that the *E.coli* was highly sensitive to Amikacin and Oxytetracycline 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 was recorded of its (fig 10).

The zone of inhibition was recorded highly sensitive to Amikacin and Oxytetracycline were (28 and 20 mm respectively) against *P. aeruginosa.* while Amoxicillin/clavulanic acid, Cefdinir, Ceftazidime, Carbeniciln and Cefuroxime had no effect of its (table 2), Compare with the highest sensitivity was recorded by Medicinal plants ethanolic extracts against *P. aeruginosa* (fig 11).

Antibiotics		Conc. Of antibiotics [µg]	Mean values of zone of inhibition (mm)	
			E.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

(Table 2): Show the effect of antibiotics against gram positive bacteria. The zone of inhibition of antibiotics was measured against *S. aureus* were Erythromycin and Norfloxacin recorded the maximum zone of inhibition was (16 and 15 mm) respectively (fig.1 and table 1), while low susceptibility was measured by Lincomycin (9mm), On the other hand Sulfisoxazole and Ampicilin had no effect on *S. aureus*.



S. epidermidis was highly sensitive to Norfloxacin where the zone of inhibition recorded as 38mm,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 13 and 14 respectively).

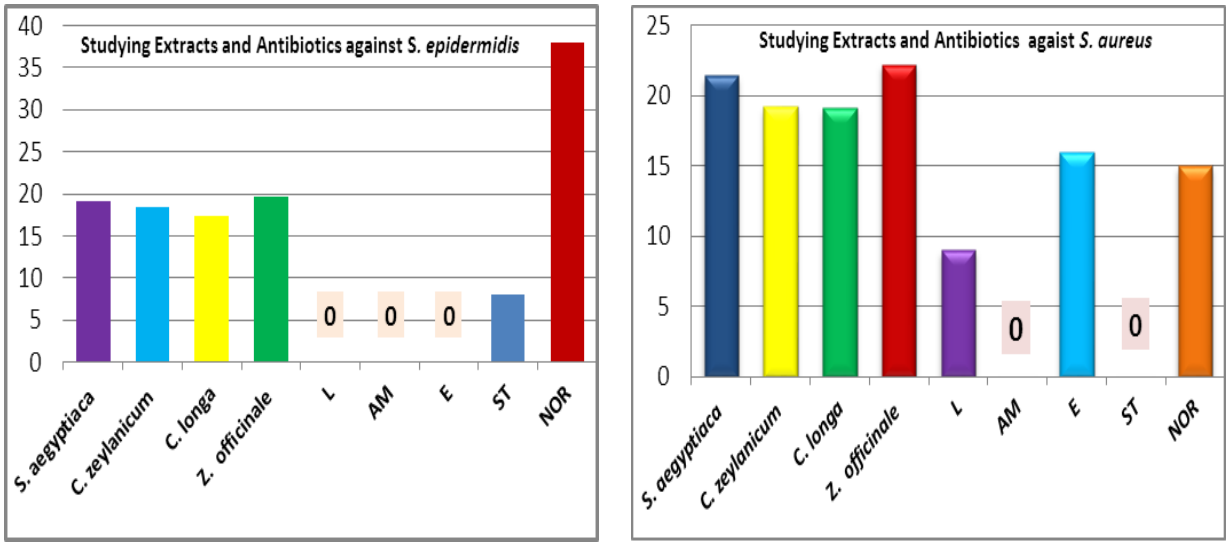
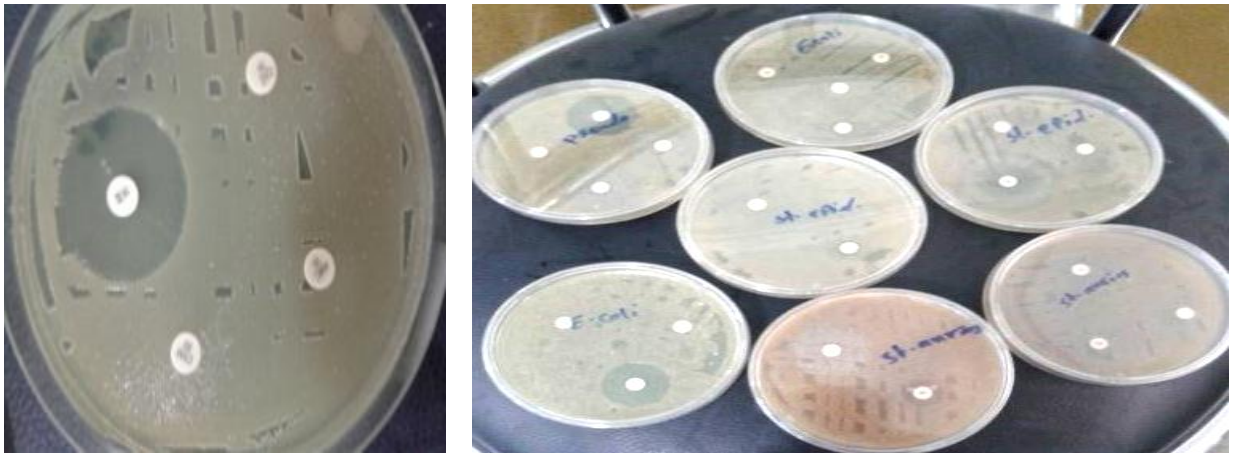
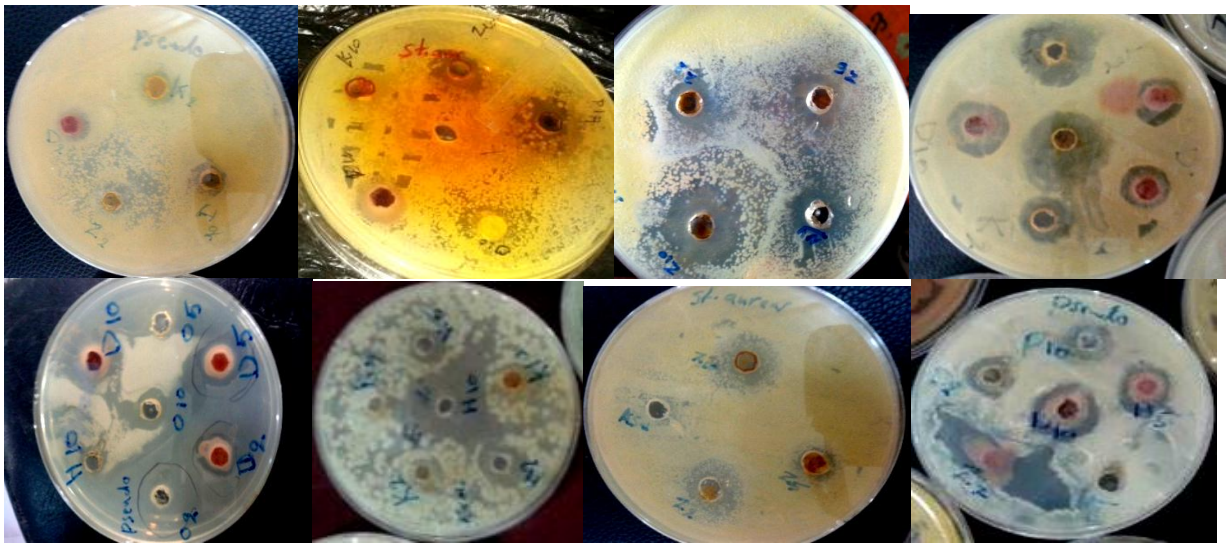


Figure 3 Effect of ethanol extracts of some antibiotics and ethanolic plant extracts on the bacteria.



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



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

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). that 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. Thus the Gram-negative outer membrane can acting as a barrier against many environmental substances, including antibiotics (Nikaido and Varara, 1985).

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.

There are several reports in the literature indicating the antibacterial and antifungal activity of the medicinal plants. 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 penetrating ability of extracts in to bacterial cells (Walsh *et al.*, 2003 and Oussalah et al., 2006).

Alcohol was found to be better solvent for extraction of antimicrobial active substances compared to water and hexanol (Ahmed *et al*., 1998). The presence of alcoholic group (-OH) in the structure of the studied ginger extracts increase the activity to inhibit the microbial growth, so the alcoholic compounds and their derivatives are considered to be antiseptic agents (Dey and Harborne, 1997),which are changing the cell protein nature and increase the permeability of cell membrane (Feeny, 1998). The differences in the antimicrobial activity of the extracts might be due to chemical composition of the plant, the species of the microorganisms used and the method of extraction. Plant originated antimicrobial drugs are of interest because in part many human and animal pathogens show multi-drug resistance and in part certain antibiotics have undesirable side effect .Further studies are needed to find out the active compounds of these plants. We concluded that, it is possible to find better therapies for many infectious diseases from the plant extracts.

CONCLUSION

Possession of useful properties, pharmacological safety make present extracts an attractive agent to explore further for its potential therapeutic applications The results of this work suggest that the compound extracted from clove and cinnamon have a broad spectrum of antimicrobial activity and this effect is increased by increasing the quantity of this compound, which can be used as an alternative for antibiotics. Therefore, pharmacological test is necessary to isolate and characterize their active compounds. Moreover, these plants extract should be investigated *in vivo* to better understand their safety, efficacy and properties. We concluded that, it is possible to find better therapies for many infectious diseases from the plant extracts.

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