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IN VITRO EVALUATION ANTIMICROBIAL ACTIVITIES OF TWO MEDICINAL PLANT EXTRACTS AND COMPARED WITH SOME ANTIBIOTICS

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ABSTRACT

The Antimicrobial properties of the ethanolic extract of three Medicinal plants, *Zingiber officinale* rhizomes and *Curcuma longa* rhizomes 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 *Zingiber officinale extracts* give higher inhibition zones 38.49 mm against all human pathogenic bacteria while the lower inhibition zones of *Curcuma longa was* 33.22 mm. Compare with The screening results of the Sensitivity test which revealed that The zone of inhibition was recorded higher sensitive to Norfloxacin was 38 mm against *S. epidermidi*s while Erythromycin, Ampicillin and Lincomycin had no effect on it .On the other hand Amoxicillin/ clavulanic acid, Cefdinir, Ceftazidime, Carbeniciln and Cefuroxime had no effect aganst *P. aeruginosa*, as well as *E.coli* was resistant against Ceftazidime, Carbeniciln and Cefuroxime. Sulfisoxazole and Ampicilin had no effect on *S. aureus*. 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: In vitro, Antimicrobial, Plant extracts, Antibiotic**.**

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, 1992; Driscoll *et al.*, 2007).

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).

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).

Zingiber officinale Roscoe (ginger, Zingiberaceae) isa medicinal herb used for treatment of various illnesses, including gastrointestinal ailments, arthritis, rheumatism, pain, muscle discomfort, cardiovascular diseases and metabolic disorders, some compounds present in the herb possess strong anti-inflammatory and antioxidative properties and exert substantial antimutagenic and anticarcinogenic activities (Shukla *et al.*, 2007 and Ali *et al.*, 2008).

Curcuma longa, or turmeric is a perennial herb andmember of the Zingiberaceae (ginger) family. The rhizome, the portion of the plant used medicinally, yields ayellow powder. In China it is ingested orally and applied topically for urticarial and skin allergy, viral hepatitis, inflammatory conditions of joints, sore throat and wounds (Kapoor, 1990). Oral administration is the main route of administration for *Curcuma longa*, it can also be used topically and viainhalation (Ayurvedic

tradition) or can be applied topicallyfor the treatment of acne, wounds, boils, bruises, blistering, ulcers, eczema, insect bites, parasitic infections, hemorrhagesand skin diseases like herpes zoster and pemphigus (WHO, 1999).

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 *Zingiber officinale* rhizomes and *Curcuma longa* rhizomes 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 for. 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 Zingiber officinale rizomes and Curcuma longa rizomes 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

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)

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. 1and 2)	Extract Weight (gram)
Zingiber officinale	Ginger	Rhizome	Pale Yellow	9
Curcuma longa	Turmeric	Rhizome	Dark Yellow	2

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.*

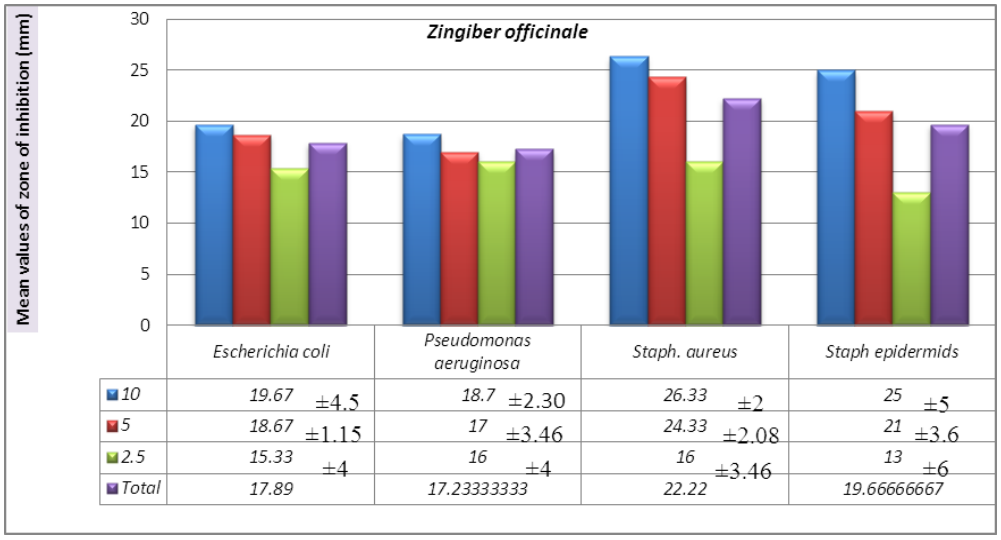


Fig. 1 shows the results of the antimicrobial screening of the crude ethanolic extract of the rhizomes of *Zingiber officinale*.

LSD.(0.05) between cons.**{E. coli = N.S;** *P.aeruginosa*=**N.S;** *Staph aureus*= **8.3 and** *S.epidermidis*= **8}**

The results show that the growth of *E. coli, P. aeruginosa, S. aureus* and *S.epidermidis* were inhibited to varying degrees by the extract. The largest zone of inhibition was produced by the 10% con. on *Staph aureus* with a zone diameter of 26.33 mm. The lowest zone of inhibition was produced by the 2.5% concentration on *S.epidermidis* which gave a zone of growth inhibition measuring 13 mm. At concentrations of 10%, 5% and 2.5% it can be seen that the extracts has antibacterial activity, where it is characterized by the presence of inhibition zone of 19.6 – 15.3 mm towards *E.coli*, 18.6–16mm towards *P.aeruginosa*, 26.33-16 mm towards the *S.aureus* and 25-13 mm towards *S.epidermidis*.

This observation due to active chemical compounds in this extract which agrees with the previous studies shown that the bioactive molecules of Z. officinale are 6-gingerol, flavonoids and phenolic acids (Ghasemzadeh *et al.*,2010).

The differences in the antimicrobial activity of the extracts might be due to chemical composition of the plant and the species of the microorganisms used. Extracts of *Z. Officinale* has been reported to posses numbers of biological activity due to major active ingredients such as zingerone, gingerdiol, zingiberene, gingeroles and shogaols, Flavonoids are the polyphenolic phytochemicals that possess a wide range of pharmacologic properties, such as antimicrobial, antiviral, anti-inflammatory, anti-allergic, analgesic, antioxidant and hepatoprotective activities, In Iraq the arial parts of *Zingiber officinale* plant are used in traditional medicine for treatment of rheumatoid arthritis and stomach ulcer (Altman and Marcussenck, 2001 and Kale et al., 2008).

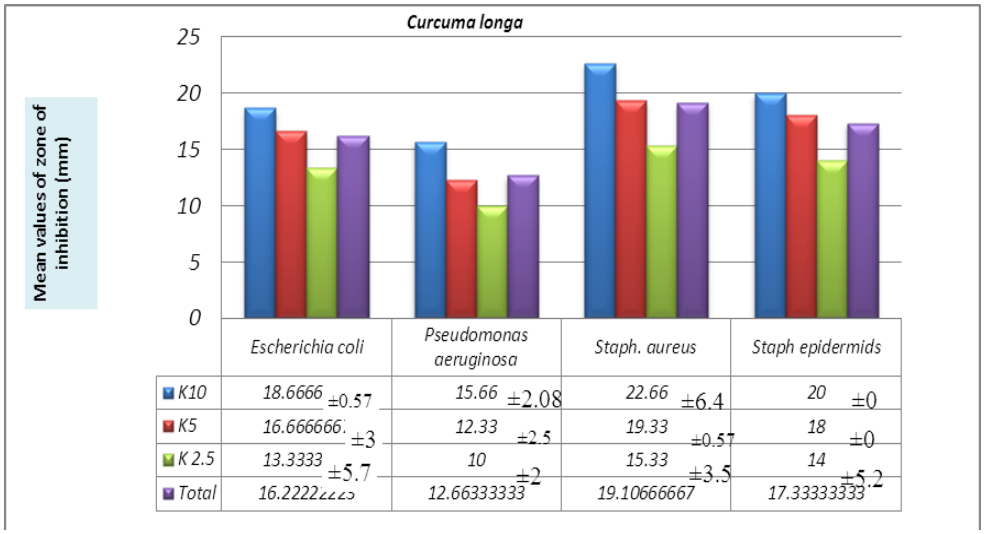


Fig. (2): Illustrates the effect of *Curcuma longa* also on *E. coli, P.aeruginosa, S. aureus* and *S.epidermidis*. It's clear that *Curcuma longa* has bactericidal effect.

It can be seen antibacterial activity of *Curcuma longa* give higher inhibition against *S. aureus* and *S.epidermidis* 22.66 mm and 20 mm respectively at 10% con. compared with *E. coli and P.aeruginosa* 18.66 and 15.66 respectively at 10% con. The lowest zone of inhibition was produced by the 2.5% concentration on *P.aeruginosa* which gave a zone of growth inhibition measuring 10 mm**.**

The results agree with studying of Negi, 1999 which shows the Curcumin has been killed several pathogenic bacteria such as Staphylococcus aureus and Enterococcus that cause infections such as skin diseases, pneumonia, meningitis and urinary tract infections in human beings. Also It has been suggested that curcumin inhibits bacterial cell division (Rai *et al.*, 2008).

LSD.(0.05)between cons.**{E. coli = N.S;** *P.aeruginosa***= 5.6;** *Staph aureus***= N.S and** *S.epidermidis***= N.S}**

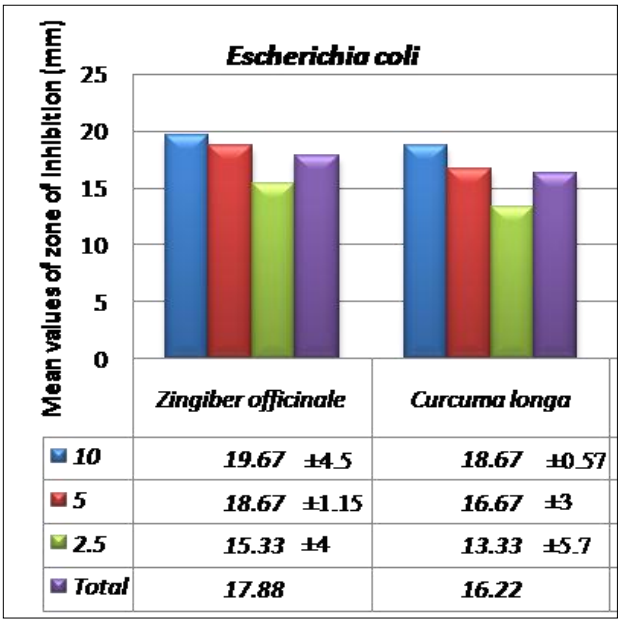
The biological and pharmacological activities of curcumin have been the subject of many reviews. This active constituents of turmeric are comprised of a group of three curcuminoids: curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin, as well as volatile oils (tumerone, atlantone and zingiberone), sugars, proteins and resins. The Curcumin is a lipophilic polyphenol that is nearly insoluble in water but is quite stable in the acidic pH of the stomach (Wang et al., 1997 and WHO, 1999).

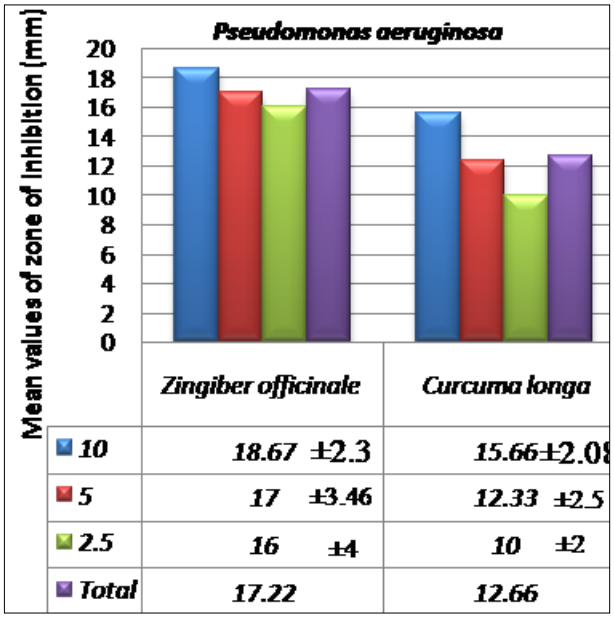
Studied the antifungal, phytotoxic, cytotoxic and insecticidal activity of an ethanolic extract of turmeric, the extract showed antifungal activity towards *Trichophyton longifusus* and *Microsporum canis*. The efficacy of curcumin or turmeric extract in reducing chemically-induced tumours was studied by (Khar *et al.*, 2001).

Fig. 5 Illustrates the effect of the *Zingiber officinale and Curcuma longa* extracts on *Escherichia coli*.

The higher mean zone of inhibition was found to be 19.67 mm at 10% con. of *Zingiber officinale* extract. While the other zones of inhibition are 18.66-13.33mm for *Curcuma longa* at 10%, 5%, 2.5% con.

(Fig. 6): Illustrates the effect of the *Zingiber officinale and Curcuma longa* extracts on *P. aeruginosa*: The higher mean zone of inhibition was found to be 18.67 mm at 10% con. of *Zingiber officinale* extract. While the other zones of inhibition are 15.66-10mm for *Curcuma longa* at 10%, 5%, 2.5% con.



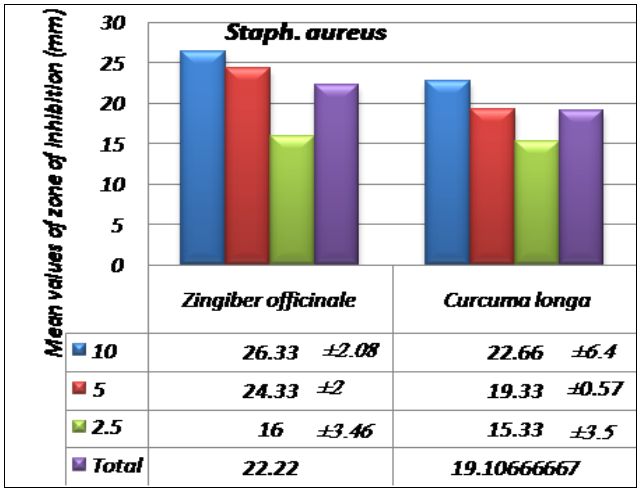


LSD.(0.05)between cons.**{***Z. officinale* **= N.S;** *C.longa* **= N.S**}

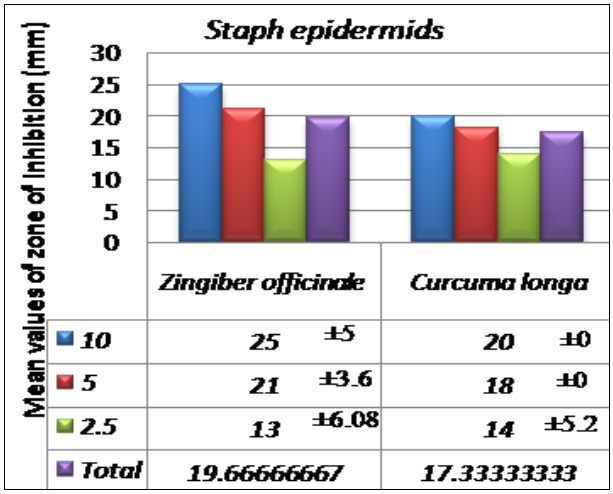
(Fig. 7): Illustrates the effect of the *Zingiber officinale and Curcuma longa* extracts on *Staph aureus*: The higher mean zone of inhibition was found to be 26.33 mm at 10% con. of *Zingiber officinale* extract . While the other zones of inhibition 22.66-15.33 mm for *Curcuma longa* at 10%, 5%, 2.5% con.

LSD.(0.05)between cons.**{***Z. officinale* **= N.S;** *C.longa* **= 5.6**}

(Fig. 8): Illustrates the effect of the *Zingiber officinale and Curcuma longa* 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 for *Curcuma longa* at 10%, 5%, 2.5% con.



LSD.(0.05)between cons.{*Z. officinale* = **8.3;** *C. longa* = **N.S**}



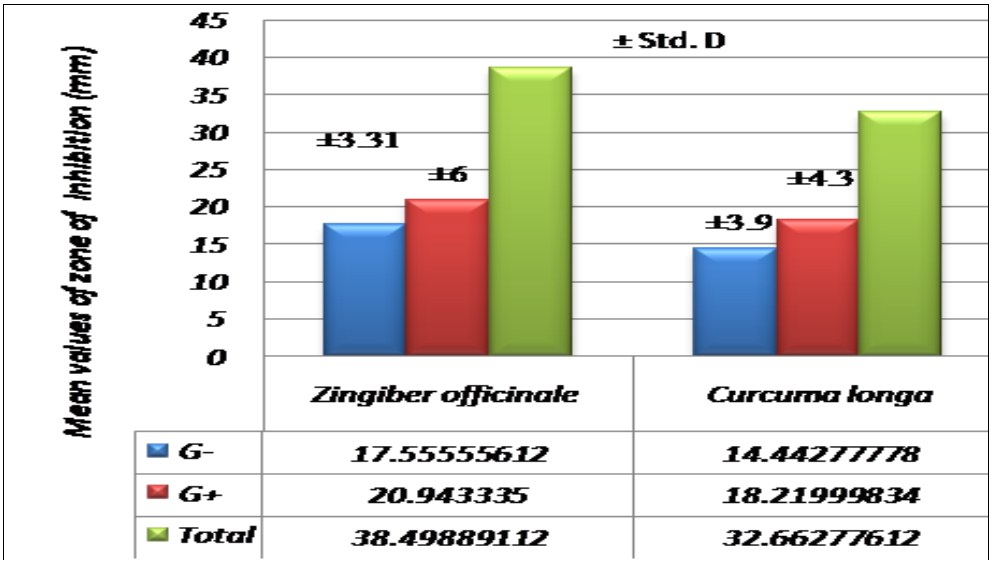
LSD.(0.05)between cons.{*Z. officinale* = **12;** *C. longa* = **5.6**}

(Fig.7): Show antibacterial activity of ethanolic extracts of the *Zingiber officinale and Curcuma longa* 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.

The results shows the higher inhibition zone of all extracts for gram positive which ranged between 20.94 mm to 18.21 mm compare with gram negative bacteria which ranged between 17.55 mm to 14.44 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 *Curcuma longa* extract 32.66mm.

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.

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). 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

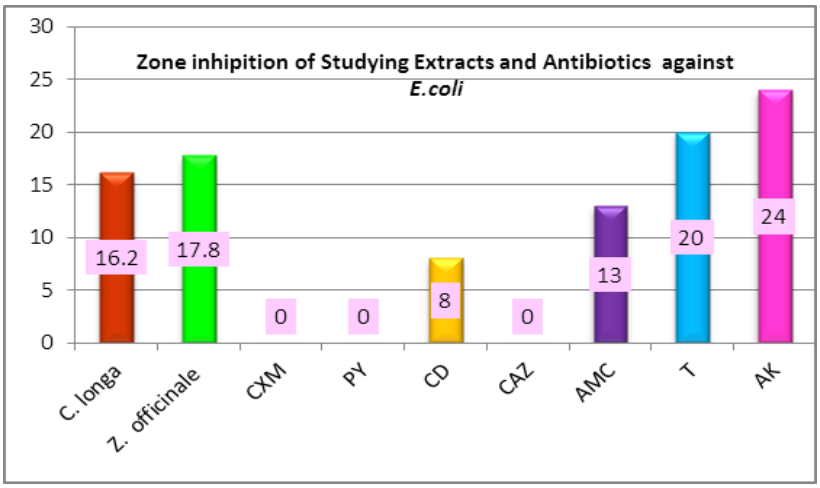
(Table 1): Show the effect of antibiotics against gram negative bacteria.

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



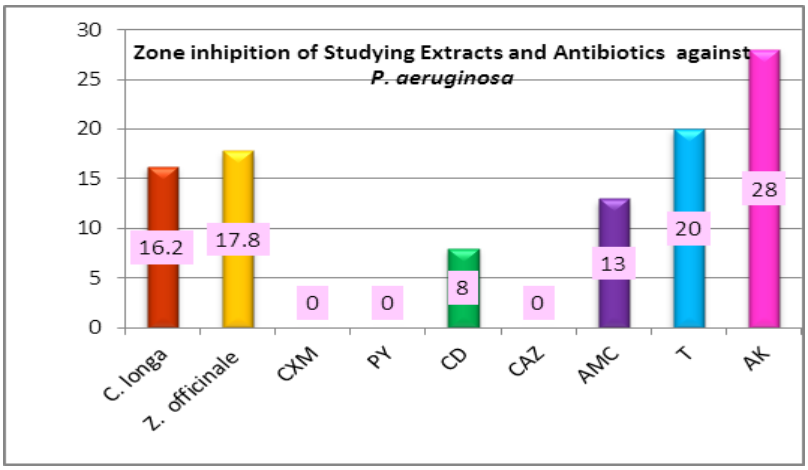
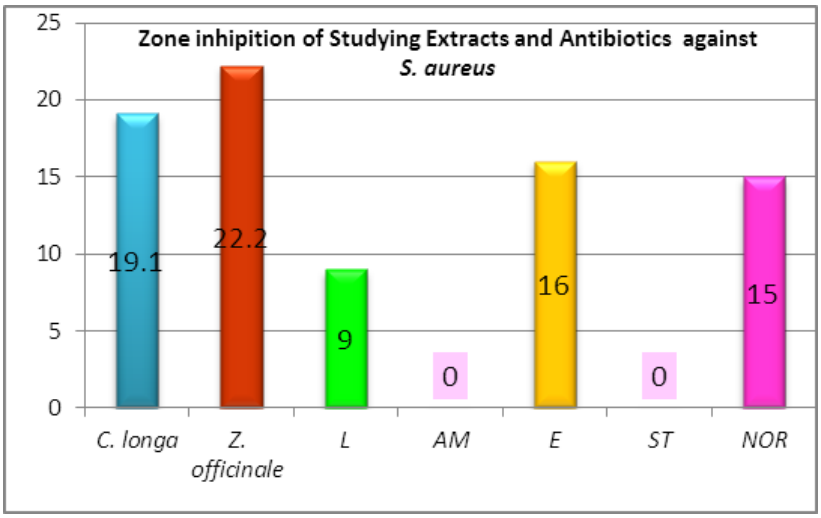


Figure 3: Effect of ethanol extracts of some antibiotics and ethanolic plant extracts on the bacterial.

(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).



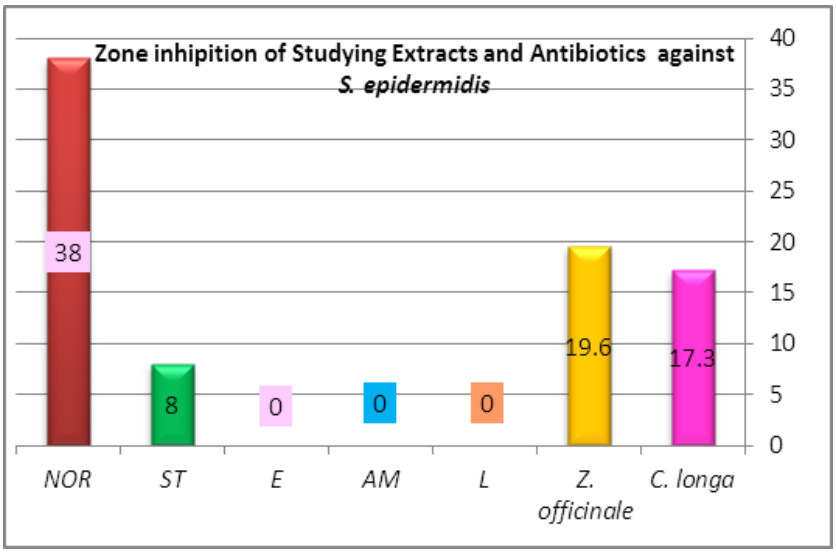
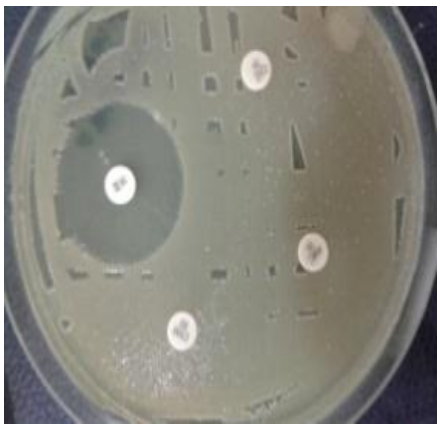
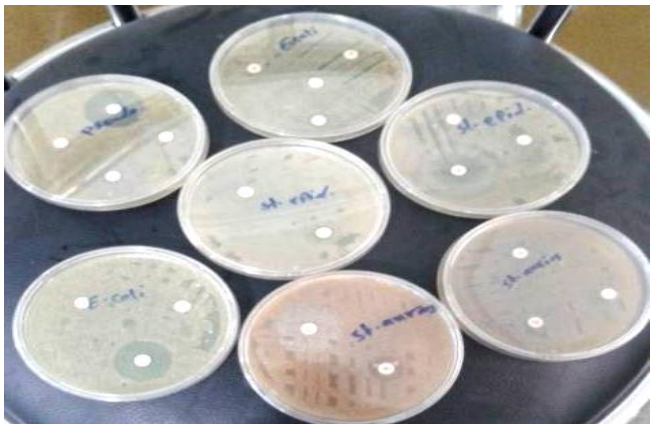
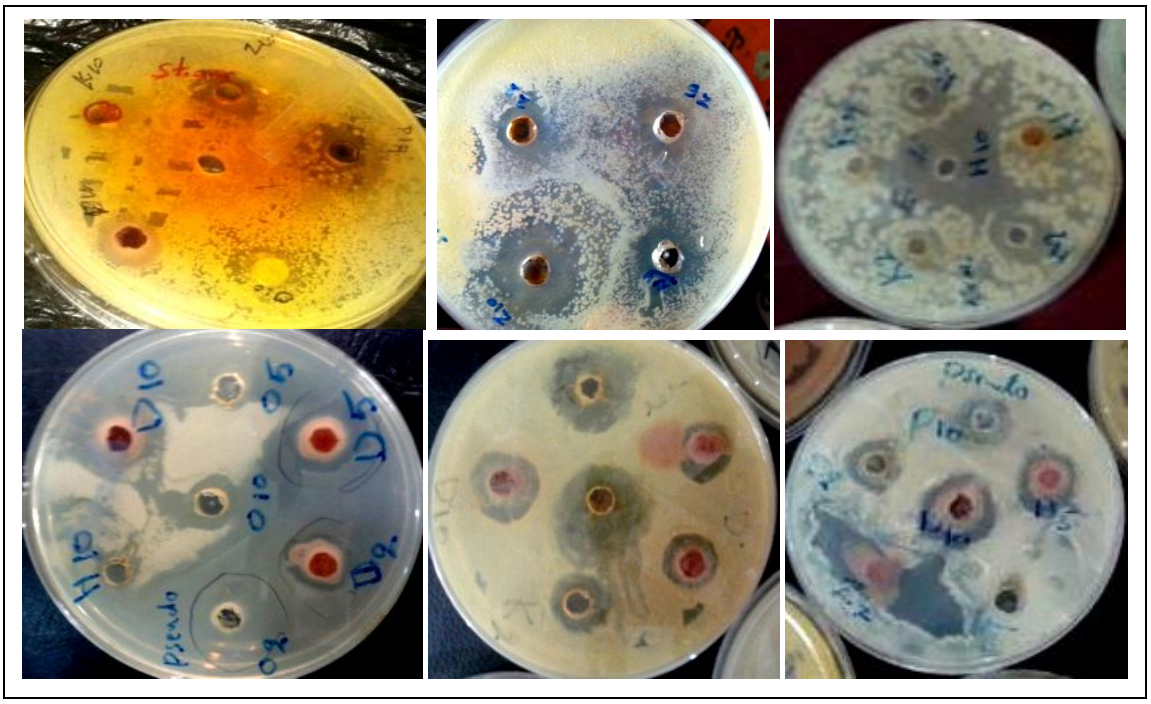


Figure 3: Effect of ethanol extracts of some antibiotics and ethanolic plant extracts on the bacteria.





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



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 that The higher resistance of Gram-negative bacteria against plant extracts is credited to the presence of outer membrane lipopolysaccharides 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. But Gram positive bacteria were more susceptible than Gram-negative bacteria by the action of extracts. 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 (Wahle *et al.*, 2010).

The differences in the antimicrobial activity of the extracts might be due to chemical composition of the plant and species of the microorganisms used and. 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 studying extracted have a broad spectrum of antimicrobial activity and this effect is increased by increasing the quantity of its, 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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