*Original Research Article*

# ASSESMENT OF ANTIBACTERIAL EFFICACY OF ETHANOLIC LEAF EXTRACTOF *EUCALYPTUS CITRIODORA*HOOK ON CLINICAL AND TYPED ISOLATES OF*ESCHERICHIA COLI*


## ABSTRACT

The emergence of some bacterial resistance to antibacterial drugs, especially to those that are easily available to local communities, has necessitated the need for discovery and development of an alternative therapy to bacterial infections. This work assessed the efficacy of the ethanolic leaf extract of *Eucalyptus citriodora* against clinical isolate and *E. coli* ATCC 35218 and compare the antibacterial activity of the extract with selected antibiotics.Standard methodswere used to determinethe antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of theextract and the inhibitory efficacy of the extract was compared with commercial antibiotics on both isolates of *E. coli* using standard method.The extract shows zones ofinhibitions of increasing concentrations. For both isolates, no zone of inhibitionswas observed at concentrations of the extract between 50-150mg/ml, but at higher concentrations between 200-500mg/ml, there were significant (P>0.05) zones of inhibitions that rages between 4.2 – 13.7 and 4.7 – 15.4mm for clinical isolate and *E. coli* ATCC 35218respectively. The susceptibility of the both isolates to conventional antibioticsrevealed ciprofloxacin (10ug) having the highest inhibition against both isolates (17.3mm and 13.9mm respectively), followed by gentamycin (14.4mm and 10.8mm), The clinical isolate was resistant to amoxicillin (30ug), while *E. coli* ATCC 35218was susceptible (4.3mm). The MIC of the extract for both isolates was 200mg/ml while the MBCs were 300 and 350mg/ml respectively*E. coli* ATCC 35218 and clinical isolate. Thecomparative zones of inhibitionsrevealed the inhibitory activitiesof the extract (15.4mm and 13.7mm) at 500mg/ml concentration could be compared favorably with ciprofloxacin(17.3mm and 13.9mm) of concentration of 10ug. Gentamycin (14.4mm and 10.8mm) could be compared favorable to concentration of the extract at 450 mg/ml (13.7mm and 11.3mm). This study revealed the potency of *E. citriodora*ethanolic leaf extract as a future herbal candidate to treat infection cause by *E. coli* at high concentrations of the extract.



Keywords: *Eucalyptus citriodora*, ,antibacterial, antibiotics resistance,*Escherichia coli*.


## 1.0 INTRODUCTION

The word *Eucalyptus* is a genus name from the Greek word *Eucalyptus*, meaning “well-covered,” and refers to its flowers that, in bud, are covered with a cup-like membrane Nair *et al.*, (1). According to Rakholiya and Chanda (2) *Eucalyptus citriodora* Hook (family: Myrtaceae) is a tall, evergreen plant which is cultivated for of essential oil, fuel, timbers andmedicinalpurposes. Husain and Ali (3), and Kharwar *et al.* (4), Reported that the leaves of *E. citriodora*produce fragrant volatile oil with antibacterial, anti-inflammatory, antiseptic, analgesic, deodorant, diuretic, expectorantactivities. The leaves contain manybioactive componentssuch as phenolic compounds, flavonoids, sesquiterpenes, aldehydes, ketones and tannins. This essential oil from this plantare widely used in cosmetics, food, and pharmaceutical industries.

Multidrug resistant (MDR) has become a public health issue, it is estimated to cause maximum deaths by the year 2050 along with increasingly high health expenses. A rise inantimicrobial resistance has been reported in *E. coli* worldwide. It’s causes complications and treatment issues (5). According to Tule and Hassani (6), *E. coli* isolates from neonates without any prior exposure to the antibiotics was highly resistant to antibiotics like ampicillin (100%) and co-trimoxazole (96%). Also, Purohit *et al*. (7), evaluated the prevalence of

antibiotic resistance of (ampicillin, cefoxitin, nalidixic acid, polymyxin-B etc.) on commensal *E. coli* isolates from human, animals, and water by disk diffusion method and reported that commensal *E. coli* from all sources displayed resistance to all the antibiotics tested except polymyxin-B. The incidence of antibiotics resistance in human isolates is higher compared that of water or animals. Nahla*et al* (8), reported an increase in multi-drug resistant phenotypes *E. coli* to third-generation cephalosporins as well as to colistin.

Walaa *et al.* (5), described *Escherichia coli* as ubiquitous microorganism that is present in both animals andenvironment. It is Gram negative,facultatively anaerobic, rod-shaped, coliformbacteria commonly found in the intestinal tract of warm blooded animals including humans. Is the most common cause of food andwater-borne human diarrhea worldwide, causing manydeaths especially in young children.It is the leading cause of urinary tract infections (UTIs),blood stream infections, wounds infections, otitis mediaand other complications in humans. More than 80% of UTIs occur in outpatients and *E. coli* accounts for more than 50% of the infections inthese patients.

Previous antibacterial studies showed that *Eucalyptus* species essential oil had antibacterial effect on the growth of Gram negative and Gram-positive bacteria. According to the report of Mounchid*et al,* (9), the antibacterial activity of *Eucalyptus* essential oils on *Escherichia coli* CIP54127 and *E. coli* isolated from urine was effective. Also, another study by Pombal *et al*. (10), reported this oil to be active against *Escherichia coli* and*Staphylococcus aureus.* This work assessed the efficacy of the ethanolic leaf extract of *Eucalyptus citriodora* against clinicalisolate and *E. coli* ATCC 35218 and compare the antibacterial activity of this extract with selected antibiotics.

## 2.0 MATERIALS AND METHODS

### 2.1 Plant leafCollection

The *E. citriodora* leafwas collected in the month of November, 2017 from Kogi State University, Anyigba, Kogi State, Nigeria, identified and authenticatedby Professor S.S. Usman, in the Department of Biological Sciences, Kogi State University, Anyigba, Kogi State, Nigeria. The voucher specimen number of the plant Bio/ FUTA/ 70.

### 2.2 Extraction and Phytochemical Screening of the leaves

The method of Dada and Oloruntola, (11) was adopted for extraction. The leaves were washed, air dried at room temperature for three weeks and pulverized using mortar and pestle. Five hundred grams (500g) of the pulverized leaf powder was macerated in 4500 ml of 75% ethanol for 72hours and then filtered using Millipore (pore size 0.7µm) filter paper. By the use of rotary evaporator at reduced temperature of $40^0$C, the filtrate was concentrated to recover the extract for further use.

### 2.3 In vitro Assay

The ethanolic leaf extract of *E. citridora* was reconstituted with dimethyl sulphoxide(DMSO) of 30% and using sterile distilled water, different concentrations were prepared as following s: 500,450,400,350, 300,250,200,150,100 and 50mg/ml (12).

### 2.4 Preparation of McFarland Turbidity Standard

This standard was prepared using the method of cheesbrought (13) to quantify the density of bacteria cell.

### 2.5 Preparation of Inoculum of *Escherichia coli* for *In vitro* Assay

The clinical isolateand *E. coli*ATCC 35218were collected from Microbiology Laboratory, University of Ibadan Teaching Hospital, Ibadan, Nigeria. The standard inocula of both *E. coli* were used for the study.

## 2.6 Antibacterial Sensitivity Test

Antibacterial activity test was carried out using the method of CLSI (14).Clinical isolate and *E. coli* ATCC 35218to the extract of the ethanolic leaf of *E. citriodora*. Using sterile pipette, 0.1ml of the bacterial suspension (1 x 106 cfu/ml) was taken and aseptically dispensed into sterile petri dishes and Mueller – Hinton Agar was poured aseptically into Petri dishes containing 0.1 ml of the suspension (1 x $10^6$cfu/ml) clinical isolate and *E. coli* ATCC 35218. The petri dishes were carefully swirled in a clockwise direction to ensure that bacteria were homogenized in MHA. The plates were left to stand for 40 minutes to solidify the medium.Using sterile cork borer of 7mm, three wells were aseptically bored on each plate at the distance of 30mm between opposite wells each and the edges of the plate. Aseptically, 0.1 ml each of the different concentrations of the extracts was then introduced into each well in the petri dishes using sterile pipette. A control well was in the center with 0.1ml of the reconstituted agents (30% DMSO). The plates were incubated at 37$^o$c for 24hours. The zones of inhibition were measured using a caliper. The study was repeated three (3) times and average value was taken, as the result of the zones of inhibition of the both isolates for different concentration of the plant extracts.

## 2.7 Antibiotic Assay

The inhibitory efficacy of the leaf extracts was compared with commercial antibiotics on both isolates of*E. coli.* Standard antibiotics(produced in England by Oxoid)such as ciprofloxacin (fluoroquinones), gentamycin(aminoglycosides), tetracycline (dexycycline),amoxicillin (aminopenicillins), ofloxacin (quinolone) andnalidixic acid (guinolone)were used against the*E. coli*. With the aid of sterile pipette 0.1 ml of the bacterial suspension (1 x $10^6$cfu/ml) was aseptically introduced into sterile plates. Sterilized MHA was aseptically poured into the plates containing 0.1ml clinical isolate and *E. coli* ATCC 35218. All the petri dishes were swirled to ensured that the bacteria weredistributed evenly in MHA, the plates were allowed to stand for 40 minutes in other to solidify the medium. Using sterile pair of forceps, the antibiotic disc was gently laid aseptically on the plate. The plate was incubated at 37$^o$c for 24 hours. All the plates were observed for zones of inhibitions around the disc. The diameter of the clear zones was measured in millimeters (mm) using a caliper.

## 2.8 Determination of Minimum Inhibitory Concentration (MIC) & Minimum Bactericidal Concentration (MBC)

Cheesbreugh (13) dilution method was adopted to determine MIC and MBC of the extract. Extract of different concentrations (500,450,400,350,300,250,200,150,100,50,25,12.5, and 6.25mg/ml) were prepared. MHA was prepared and 5ml was pipetted into sterile test tube and 0.1ml of inoculum of *E. coli* (1 x $10^6$ cell/ml) was introduced into each test tube and was properly mixed.With the aid of sterile pipette, 1ml of the various concentration of extract prepared was dropped into each test tube containing the broth culture clinical isolate and *E. coli* ATCC 35218.The mixture was incubated at 37$^o$c for 24hours. Turbidity measurement using a spectrophotometer was checked for growth in each test tube. High turbidity indicated growth andinhibition of growth was indicated by low turbidity.The concentration in which no growth was observed as shown by cleared broth indicated the minimum inhibitory concentration while the MBC was determined by taking a loopful each from test tube that showed no growth during MIC assay and streaked on agar plate that is free of leaf extract, incubated at 37$^o$c for 24 hours. The least concentration at which no growth as observed was noted as the MBC.

## 2.9 Statistical analysics

All datawereexpressedasmean±S.E.One-wayanalysisofvariance was used toanalyze data. $P<0.05$wasconsideredsignificantdifference betweenmeans(Duncan'smultiplerangetest).

## 3.0 Results

### 3.1 Percentage Yield of the Ethanolic Leaf Extract of *Eucalyptus citriodora*

Percentage yield of the ethanolic leaf extract of *Eucalyptus citriodora* was 9.37% (46.83/500g) (Table 1).

### 3.1 Antibacterial Activity of Ethanolic Leaf Extract of *E. citriodora*

The sensitivity pattern of clinical isolate and *E. coli* ATCC 35218to ethanolic leaf extract of *E. citriodora*(figure1),revealedan inhibitory effect of increasing concentrations of the extract. For both isolates, no zone of inhibition was observed at concentrations of the extract between 50-150mg/ml, but at higher concentrations between 200-500mg/ml, there was a significant increased (P<0.05) in zones of inhibitions that rages between 4.2 – 13.7 and 4.7 – 15.4mm for clinical isolate and *E. coli* 35218respectively.

### 3.2 Antibiotics Sensitivity Pattern of *E. coli*

Thesensitivity test of*E. coli* 35218and clinical isolatesto conventional antibiotics(figure 2), revealed that,ciprofloxacin (10ug) had the highest zones of inhibitionsagainst E. *coli* 35218and clinical isolates(17.3mm and 13.9mm respectfully), followed by gentamycin (14.4mm and 10.8mm), tetracycline (13.9mm and 7.8 mm), ofloxacin (13.0mm and 8.3mm), amoxicillin (4.3mm and0.0mm) and nalidixic acid (10.8mm and 5.4 mm). However, clinical isolate was resistant to amoxicillin (30ug), while *E. coli* 35218was susceptible (4.3mm).

### 3.3 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of the extract for *E. coli* ATCC 35218and clinical isolatewas 200mg/ml while the MBCs were 300 and 350mg/ml respectively for *E. coli* ATCC 35218and clinical isolate.

Table 1: Percentage yield of ethanolic leaf extract of *Eucalyptus citriodora*

| Plant species | Plant part | Weight of powder (g) | Volume of Solvent (ml) | Yield (g) | % Yield |
|---|---|---|---|---|---|
| *E. citridora* | Leaf | 500 | 4000 | 46.83 | 9.37 |

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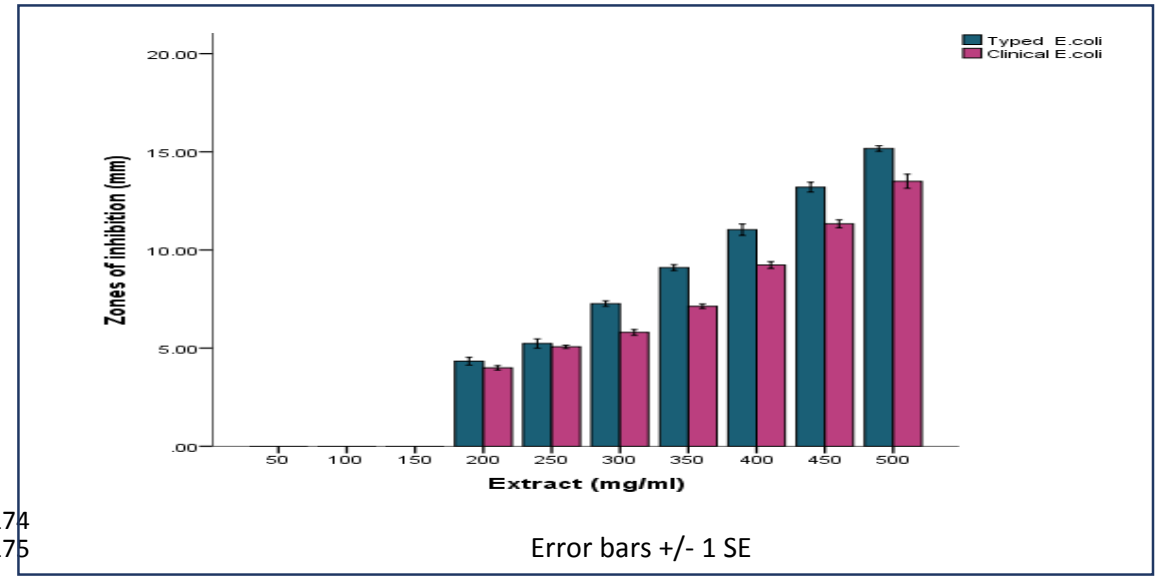


**Figure 1: Antibacterial activities of*E.coli* ATCC 35218and clinical isolateto the extract.**

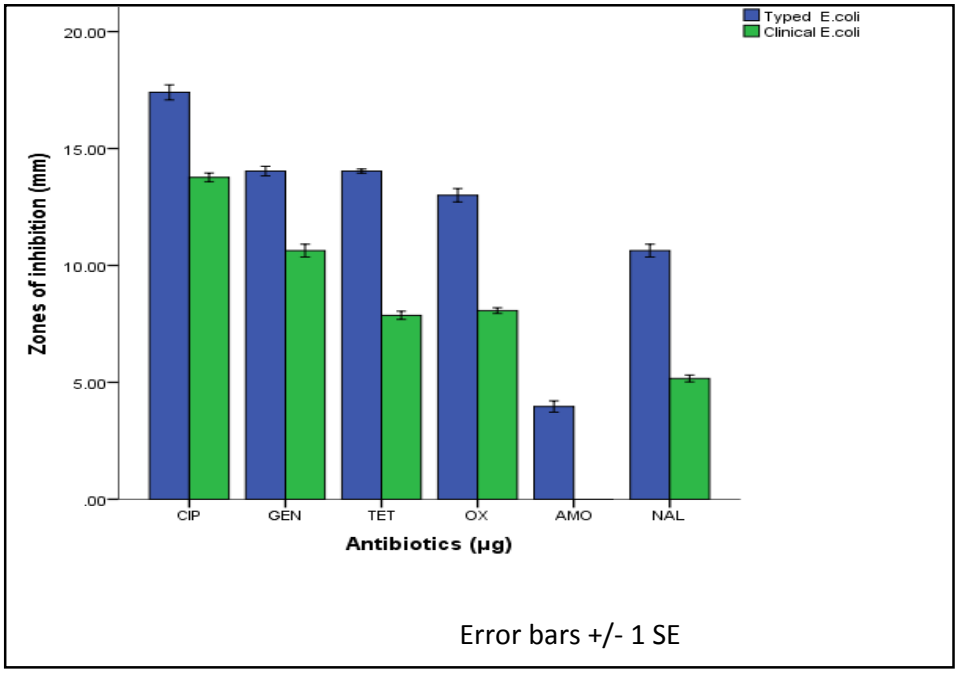


**Figure 2: Sensitivity of*E. coli* ATCC 35218and clinical isolateto commercial antibiotics.**

### 3.4 Comparative Zones of Inhibition of Conventional Antibiotic with *E. citiodora* Leaf Crude Extract

The result of the comparative zones of inhibitions of the conventional antibiotics with the extract(figure3) revealed that, in both *E. coli* ATCC 35218and clinical isolate, the inhibitory activitiesof the extract (15.4mm and 13.7mm) at 500mg/ml concentration could be compared favorably with ciprofloxacin of concentration of 10ug (17.3mm and 13.9mm). Gentamycin (14.4mm and 10.8mm) could be compared favorable to concentration of the extract at 450 mg/ml (13.7mm and 11.3mm), tetracycline (13.9mm) and ofloxacin (13.0mm) could be compared with the concentration of the extract at 450mg/ml(13.7 mm) for *E.coli* ATCC

35218. While tetracycline (7.8mm) and ofloxacin(8.3mm) could be compared with extract concentrations of 350 and 400mg/ml (7.2mm and 9.3mmrespectfully) for clinical isolate. Amoxicillin (4.3mm) can be favorably compared to extract at the concentration of 200mg/ml (4.7mm) for *E. coli* 35218. Nalidixic acid (10.8mm) could be compared to extract concentration at 400mg/ml (11.1mm) for *E. coli* ATCC 35218, while for clinical isolate, nalidixic acid (5.4mm) is compared favorable to extract concentration of250mg/ml (5.1mm).

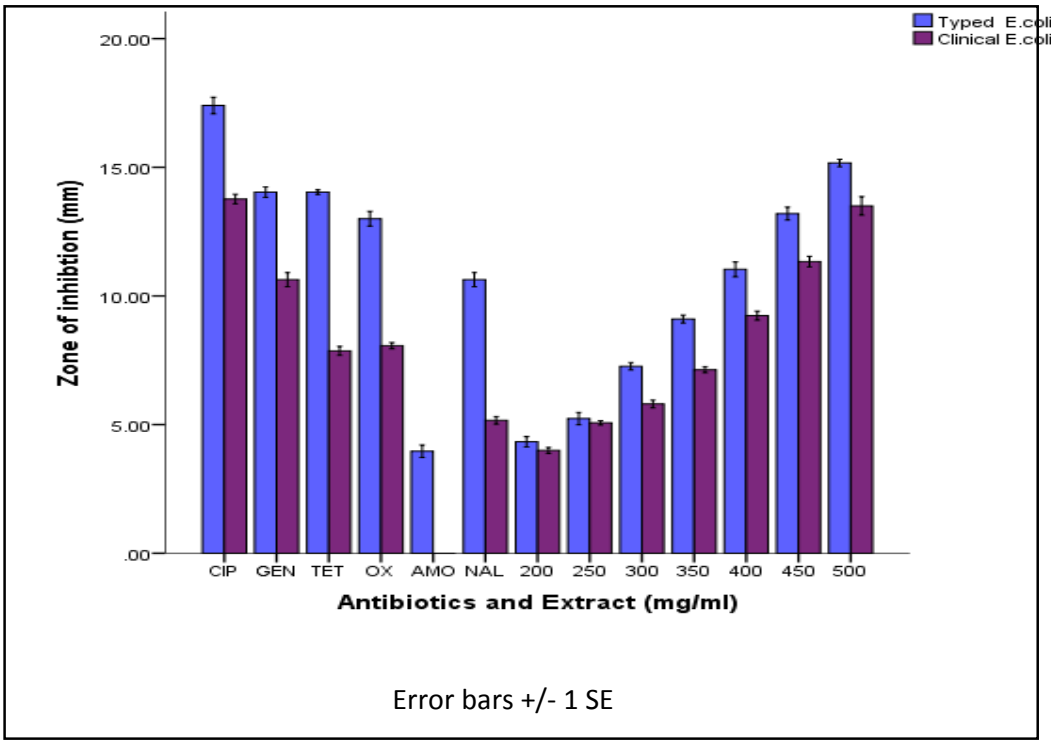


Figure 3: Comparative study of the Susceptibility of the clinical isolate and *E. coli* 35218to the plant extract and commercial antibiotic

## 4.0 DISCUSSION

The inhibitory pattern of clinical isolate and *E. coli* 35218by ethanolic leaf extract of *E. citriodora* varied from concentrations to concentrations. The extract of *E. citriodora*displayed antibacterial activities against both isolates of *E. coli*, but at high concentrations. This agreedto similar report ofTolba*et al*. (15), that, the zones of inhibitionsof *E. citriodora* oil extract increases with increase concentrations and that *E. coli*, was extremely sensitive to the oil extract (26 ± 0.0 mm). Thezonesinhibitions observed might probably be due to report advanced byEvans (16), that alkaloids occur in plants in association with characteristic acids.This acid could be probably responsible for the zones of inhibitions observed.

Lack of inhibitions observed on bothisolates at low concentration (50-150mg/ml) could be due to other bioactive components not tested in this study that could be absent in the extract.This corroborated with the report of Tolba*et al*. (15),who stated that antibacterial activity of many essential oils, and in particular *Eucalyptus* species, is related to the presence of some compounds such as alcohols, aldehydes, alkenes, esters.Also, antimicrobial activity of the *Eucalyptus citriodora*Hk essential oil could be due to the two major compounds: citronellal and citronellol. However, the zones of inhibitions observed at high concentrations might be due to presence of tannins and other bioactive components in the extract. This agrees with the report advanced by Dickson *et al*. (17), that the presence of tannins in plant suggest its medicinal value, because tannins have potential antiviral, antibacterial and antiparasitic effects. This also agreedwith the report of Amabye*et al*. (18),

that tannins are known to be made up of phenolic compounds and phenols have been used extensively as disinfectants and action of tanninsmight be due to protein denaturation. The lack of inhibitions observed at low concentrations could suggest that infection caused by *E. coli* might not be treated with low concentration of *E. citriodora* extract. This disagreed with Dickson*et al*. (17), who reported that at low concentrations (≥50mg/ml), the aqueous extract of *Eucalyptus* might be effective in the treatment of diseases caused by virulent strains of *E. coli*.Clinical isolate was more resistant to the plant extract comparedto *E. coli* 35218. This resistant could be due to report advanced by Yaya *et al* (19), thatthe membrane of this strain was impervious to the active components contained in the extract at those concentrations. Also, lipopolysaccharides and phospholipids cell wall of the isolate, could block the penetration of the extract inside the cell cytoplasm.

The obtained values of MIC and MBC for both isolates was higher than that reported by Luqman*et al.* (20). Also, Tyagi and Malik, (21),reported low value of MIC for *Eucalyptus globulus*(4.5 mg/ml) on*E. coli*.

The antibacterial sensitivity of both isolates to ciprofloxacin, ofloxacin, nalidixic acid, tetracycline and gentamycin is unexpected and this might probably due to non-indiscriminate previously exposure of this strain of *E. coli* to those antibiotics. This disagrees with the work of Lucia *et al*. (22), who observed resistanceof*E. coli* strain to these antibiotics. With the exception of gentamycin that displayed inhibition (11.4mm). The sensitivities of the isolates to ciprofloxacin and gentamycin are expected, this agreed with the similar result advanced by Ahmed *et al*. (23), that ciprofloxacin and gentamicin revealed high sensitivities against *E. coli* isolates with 80 and 66.66% sensitivity respectively, these sensitivities were higher than that of the current study and this might probably be due to the strain of *E. coli* involved. This also agreed with the report of Reuben and Owuna (24), that 78.9% of *E. coli* isolates displayed sensitivity to ciprofloxacin and same percentage was observed for gentamicin. The susceptibility displayed by *E. coli* isolates to ciprofloxacin and gentamicin in this study suggested their effectiveness in the treatment of infections caused by *E. coli*. The sensitivity of *E. coli* isolates to tetracycline in this study was unexpected been the most commonly prescribed antibiotic in the hospital and also the most easily available in the communities without prescription. This isdisagreedwith the report of Reta *et al.* (25). The resistant recorded for amoxicillin in this study agreed with the report of Kindu (26). This is expected because of easy accessibility and low cost of the antibiotic and this resistant could also probably due to reasons advanced by Todar, (27),that antibiotics resistance develops when microorganisms are exposed to effective doses of antibiotics within a shorter period or when the organisms are exposed to smaller concentrations of the antibiotics over a longer period of time.According toAbdel-Rahman*et al*. (28), DMSO have no antimicrobial activity against this test organisms, that is why we considered it as control in the analysis.

Findings from comparative zones of inhibitions of the extract to antibiotics revealed that, in both isolates, the concentration of the extract at 500mg/mlwhich show highest zones of inhibition can be compared favorably with ciprofloxacin that also shows highest inhibition. Similarly, the least inhibition displayed by the extract at concentration of 200mg/ml can be compare favorably with amoxicillin that displayed lowest activity.

### 4.1 Conclusion

This study provided information on the future herbal potency of *E. citriodora*leaf extract as a candidate for treatment of *E. coli* infection.Further investigation to determine the pure active components of the leaves extract of the *E. citriodora*responsible for these activities and the effect on long term administration is recommended for further studies.

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