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COMPARATIVE STUDIES OF ANTIBACTERIAL ACTIVITIES OF SELECTED LOCAL SPICES ON SOME CLINICAL ISOLATES

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ABSTRACT

Keywords:

Antibacterial activity, African black pepper (Piper guineense), clove (Eugenia caryophyllum), Antibiotics, ginger (Zingiber), garlic (Allium sativum), nutmeg (Myristica fragrans).

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Spices are produced from leaves, barks, bulbs, stems, flowers and seeds of certain plants and mostly grown and harvested in the warm humid areas of the world. They are used for aroma, taste, color, and preservation of food and beverages. Selected spices used in the study were clove, ginger, garlic, black pepper and nutmeg. They were extracted via percolation using ethanol and water. The extracts were tested for antibacterial activities against two clinical isolates (*Staphylococcus aureus* and *Escherichia coli*) using well diffusion method. Clove showed the highest antibacterial activity at all concentrations, having 35mm zone of inhibition at 1000 mg/100ml. Black pepper had the lowest antibacterial activity of 16mm zone of inhibition at 1000 mg/100ml concentration and also showed the least zone of inhibition compared to the other four tested spices. Also garlic, ginger showed positive effect on the clinical isolates whereas Nutmeg required a very high concentration to be effective. Antibiotics are still effective on the clinical isolates, at 100% concentration, ciprofloxacin had 30 mm zone of inhibition on *Escherichia coli*, nitrofurantoin have 26mm zone of inhibition on *Staphylococcus aureus*, while tetracycline is less effective having 14mm zone of inhibition. This study revealed that spice extracts are more effective than antibiotics. Comparative studies using antibiotics showed that clove, ciprofloxacin and garlic are active against the bacteria used in this study. This was attributed to similarity in the mechanism of action of garlic and ciprofloxacin. Some advantages of these spices are enhancement of organoleptic and preservative properties which can be used as food additives to control growth of food borne pathogen.

INTRODUCTION

Spices constitute an important group of agricultural commodities, which are virtually indispensable in the culinary art. The spices are produced from roots, barks, leaves, bulbs, stems, flowers and seeds of

certain plants and mostly grown and harvested in warm humid areas of the world. Spices are used for flavor, color, aroma, taste and preservation of food and beverages.

Spices are often dried and used in a processed but complete state. Another option is to prepare extracts such as essential oils by distilling the raw spice material (wet or dry) or to use solvents to extract oleoresins and other standardized products (Moreira, 2009).

Many foods have antibiotic functions that are often unknown to the consumer which reduced or limited the growth of bacteria in their body. In fact, the use of medicinal plant to treat disease of varying etiology is part of the Africa tradition, but in spite of thousands of years of use, none of these bio-active plants compounds have been exploited for clinical uses as antibiotics, though some alkaloid compounds like quinine and emetine have been developed as chemotherapeutic agents among those antibacterial foods that are becoming more common in western diet are ginger, garlic, *Eugenia cinnamomum*, *Piper guineense* and nutmeg.

Antibiotics are chemicals produced by micro-organisms. Micro-organisms that produce antibiotics are mostly bacteria. These five named spices (ginger, garlic, eugenia, piper guineense and nutmeg) have been valued for its antibacterial properties for thousands of years in Asian cultures (Weil, 2005).

Ginger (*Zingiber*) is one of those plants that were seriously investigated over the years it has been used in centuries to fight infection, most especially diarrhea which is a leading cause of infant death in developing countries and tearing nausea caused by sea sickness, morning sickness (Hoffman, 2007).

In in-vitro studies, Garlic has been found to have antibacterial, antiviral and antifungal activity. Garlic is also claimed to help prevent heart disease (including atherosclerosis, high cholesterol and high blood pressure) and cancer. Garlic is used to prevent certain types of cancer

including stomach and colon cancers. In fact, countries where garlic is consumed in higher amounts because of traditional cuisine have been found to have a lower prevalence of cancer. The known vasodilative effect of garlic is possibly caused by catabolism of garlic derived polysulfides to hydrogen sulfide in red blood cells (RBC) (Durak *et al.*, 2006).

The Nutmeg tree is any of several species of trees in genus *Myristica*. The most important commercial spices species is *Myristica fragrans*, (Nathaniels, 2008). Nutmeg is also popular for its many health benefits. It is used when suffering from muscle pain, arthritis sores and other problems to relieve the pain, apply Nutmeg oil to the affected areas and ginger too is used for these purposes. In a 2010 study, daily consumption of Ginger was shown to help ease muscle pain associated with exercise by 25% and have blood thinning and cholesterol lowering properties advanced glycation end-products are possibly associated in the development of diabetic cataract for which ginger was effective in preliminary studies apparently by acting through antinglycating mechanism (Afshari, 2007). *Zingerone* may have activity against enterotoxigenic *Escherichia coli* in enterotoxin-induced diarrhoea.

Eugenia and *Piper guineense* commonly referred to as African black pepper or Ashanti pepper; is very similar to *Piper nigrum*. *Piper guineense* as a plant has been reported to have medicinal cosmetic (Dalziel, 1995) and insecticidal properties. *Eugenia* is used to improve the healthy gum and prevent from tooth decay.

The research is thus aimed at investigating the effect of some local spice extracts, (ginger, garlic eugenia, piper guineense and nutmeg) as antibacterial agent on some clinical isolates.

MATERIALS AND METHODS

Collection of Samples

Fresh Ginger, Garlic (*Allium Sativum*), Clove, Piper guineense and Nutmeg were purchased from the local market of yan'gwari Rigasa Kaduna. The spices were cleaned, descaled where necessary and washed using sterile distilled water.

Preparation of Ethanol and Aqueous Extract

Ethanol and distilled water were used as solvent for the extraction of plant materials. Ten (10g), twenty five (25g), fifty (50g), seventy five (75g) and hundred grams (100g) of ginger powder, squeezed fresh garlic bulbs, Nutmeg, Clove powdered and powdered black pepper were weighed separately using a clean electronic weighing balance and then poured into a sterile bottle jar containing 100ml of distilled water and 100 ml 95% ethanol (Silva *et al.*, 1999). The bottle jars were labeled appropriately to avoid misplacement. For ginger, nutmeg and clove, the mixture were stirred and allowed to stand each for 24hrs and filtered using sterile Whittman no 1 filter paper, the filtrate was concentrated to 20ml on a water bath and evaporated to dryness at room temperature. The extracts obtained were used for the analysis of antibacterial activities using well diffusion method (Weil *et al.*, 2005).

Phytochemical Screening

A qualitative phytochemical test to detect the presence of alkaloids, carbohydrates, flavonoids, Resins, saponins, steroids and tannins was carried out on all the extracts using standard methods.

a. Test for Carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with

2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars (Prashant *et al.*, 2011).

b. Test for Saponins (Frothing test)

In this test, half gram (0.5g) of each extract was placed in a test tube and then 0.5ml of distilled water was added. The tube was then shaken vigorously. A persistent froth that lasted for at least 15mins indicated the presence of saponins (Shamsuddeen *et al.*, 2009).

c. Test for Tannin

Solutions of the extracts were made with distilled water and 3 drops of 5% ferric chloride solution was added. A green-black or blue-black colouration indicated the presence of tannins (Shamsuddeen *et al.*, 2009).

d. Test for steroids: Salkowski Test:

To 2ml of aqueous extract, 2ml of chloroform and 2ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence (Harsha *et al.*, 2013).

e. Test for Reducing Sugar

One milliliter (1ml) of stock solution of each extract was diluted with 2ml of distilled water, followed by the addition of Fehling's solution (A+B) and the mixtures warmed. Brick red precipitate at the bottom of the test tubes indicated the presence of reducing sugars (Shamsuddeen *et al.*, 2009).

f. Test for Flavonoids

With Lead acetate - To small quantity of aqueous extract 10% of lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

With Sodium Hydroxide- On addition of an increasing amount of sodium hydroxide, the aqueous extract showed yellow coloration, this decolorized after addition of acid (Harsha et al., 2013).

g. Test for Alkaloids

The aqueous extract was evaporated in a test tube. To the residue dilute HCl was added, shaken well and filtered. With the filtrate following tests were performed.

Hager's Test- To the 2-3ml of filtrate hager's reagent was added. Yellow precipitate was formed showing the presence of alkaloids.

Mayer's Test- To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids.

With tannic acid- To 1ml of extract add 2-3 drops of the tannic acid solution reagent, appearance of amorphous or crystalline precipitate represents the presence of alkaloid (Harsha et al., 2013).

h. Test for Glycosides - Borntrager's Test:

To the 3ml of aqueous extract, dil. H₂SO₄ was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. This solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides (Harsha et al., 2013).

i. Test for Resins

One ml of various solvent extracts was treated with few drops of acetic anhydride solution followed by one ml of conc.H₂SO₄. Resins give coloration

ranging from orange to yellow (Diksa et al., 2013).

Confirmatory Microbiological Analyses

The clinical isolates (*Staphylococcus aureus* and *Escherichia coli*) collected from Yusuf Dantsoho Memory Hospital were further subjected to microbiological analyses for confirmation.

a. Catalase test

Drops of 3% of hydrogen peroxide were placed on a glass slide. A bit of growth from a solid medium was removed with a wire loop. Bubbling was formed which indicated that the organism is Catalase positive (Cheesbrough, 2003).

b. Indole Test

A loopful of organism was inoculated in 5ml of peptone water and it was incubated at 37°C for 24hours. 3-8 drops of kovacs indole reagent was added and shaken gently. A red colour in the reagent layer appeared above the broth within 1 minute (Cheesbrough, 2003).

c. Motility Test

Organism was inoculated in nutrient agar by making a stab using a needle to a depth of 1-2cm short of the bottom of the tube. It was incubated at 35°C for 24-48hours. At the end of the period of incubation, the line of inoculation was sharply defined and the rest of the medium was somewhat cloudy which indicates that the organism is motile (Cheesbrough, 2003).

d. Urease Test

The organism was streaked on urea agar slant in bijou bottle and it was incubated at 37°C for 24-72hrs. A positive test showed bright pink colour appearance (Cheesbrough, 2003).

e. Citrate Utilization Test

Organism was streaked on a simmon citrate agar slant in a bijou bottle and it incubated at 30°C for 24hrs, a positive test showed deep blue color appeared (Cheesbrough, 2006).

f. Triple Sugar Iron Agar (TSI) Test

The organism was streaked on the surface of the agar slant; it was incubated at 35°C for 24hrs. A gas was formed; it was determined by the appearance of several bubbles (Cheesbrough, 2006).

g. Coagulase Test

Two drops of physiological saline was dropped about 2cm apart on a clean glass slide that has been divided into two with a marker. One colony from a culture was emulsified on each drop of saline. A loopful of citrated human plasma was added to the bacterial suspension and mixed with the loop. The slide was rocked gently for 1 minute. Clumping or agglutination of cells was observed which shows coagulase positive reaction (Cheesbrough, 2006)

ANTIBACTERIAL SUSCEPTIBILITY TEST

Agar diffusion susceptibility test

Two loopfuls of the standard inoculum were evenly streaked on to the plates in duplicates. Discs of different concentrations as well as the control discs (impregnated with only diluent) were placed firmly on the surface of the medium (Mueller Hinton agar) by means of sterile syringe needle at about 40mm apart.

The plates were incubated at 37°C for 24 hours. Diameters of the zones of inhibitions were measured with ruler and the mean recorded to the nearest (mm) (Shamsuddeen *et al.*, 2009).

a. Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the potent extracts were determined according to the macro broth dilution technique (Mbata and Saikia, 2007). Confirmed *Staphylococcus aureus*

and *Echerichia coli* were inoculated into a series of sterile tubes of nutrient broth containing two-fold dilutions of the spices extracts and incubated at 37°C for 24 hours. After incubation, tubes were examined for presence or absence of growth of organism using turbidity as criteria. The turbidity was visually compared with 0.5 of Mcfarland standard, (National Committee for Clinical Standard, 2003). The minimal inhibitory concentrations (MICs) were read as the least concentration that inhibited the growth of the test organisms (Mbata and Saikia, 2007).

b. Minimum Bacteriicidal Concentration (MBC)

This was determined by plating out the content of Minimum Inhibitory Concentration tube which shows no growth or turbidity: a loopful from each tube was subcultured onto extract free agar plates, and incubation for 24hours at 37°C. The least concentration, at which no growth was observed, was noted as the MBC (National Committee for Clinical Standard, 2003).

RESULTS

Figure 1: Showed clove to have the highest antibacterial activity at all concentration, having 35mm zone of inhibition at 1000 mg/100ml. Black pepper had the lowest antibacterial activity of 16mm zone of inhibition at 1000 mg/100ml concentration, while there is no any effect at 500, 250 and 100mg. Also garlic showed positive effect on the clinical isolate with 34 mm zone at 1000mg/100ml concentration and ginger 30mm zone of inhibition. Nutmeg is only effective at 1000mg and 750 with a zone of inhibition of 18mm and 12mm respectively.

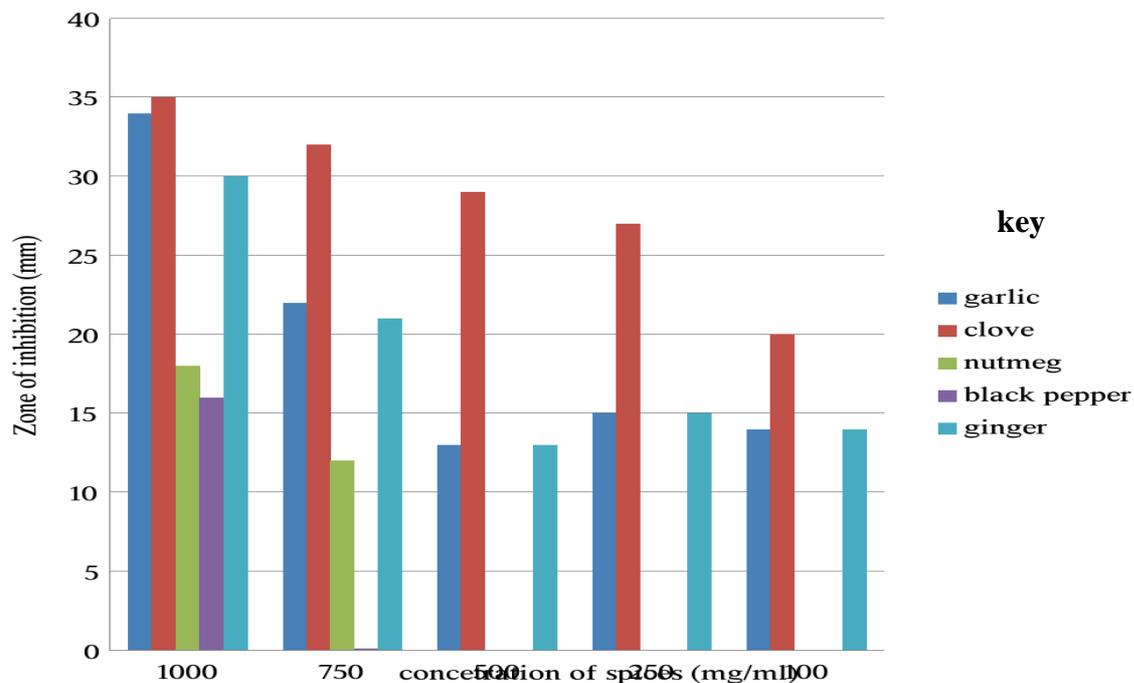


Fig. 1 Effect of ethanol spices extract on *staphylococcus aureus*

Figure 2 showed that clove had the highest antibacterial activity at all concentration, having 29mm zone of inhibition at 1000mg. Black pepper had the lowest antibacterial activity of 16 mm zone of inhibition at 1000 mg concentration, while there is no any effect at 500, 250 and 100mg. Garlic also showed positive effect

on the clinical isolate. With 28mm zone of inhibition at 1000mg concentration and ginger 26mm zone of inhibition. Nutmeg requires a very high concentration whereby the spice is only effective at 1000 and 750mg with a zone of inhibition of 13mm and 10mm respectively.

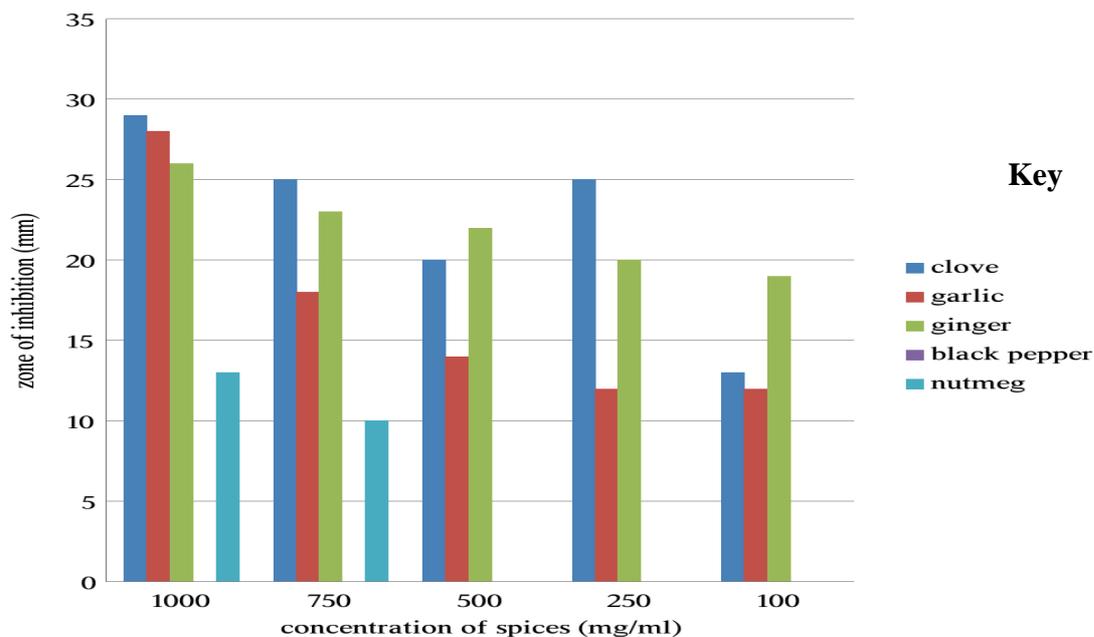


Fig 2.Effect of ethanol spices extract on *Escherichia coli*

Figure 3 showed that clove has the highest zone of inhibition of 28 mm in 100% and 20 mm in 75% while *piper guineese* had the lowest zone of inhibition of 10mm at 1000mg and 8mm at 750mg/100ml. Also

garlic and ginger are active at 1000mg and 750mg/ml with zone of inhibition of 28mm and 22mm. Black pepper is only active at 1000mg/100ml with a zone of inhibition of about 12mm.

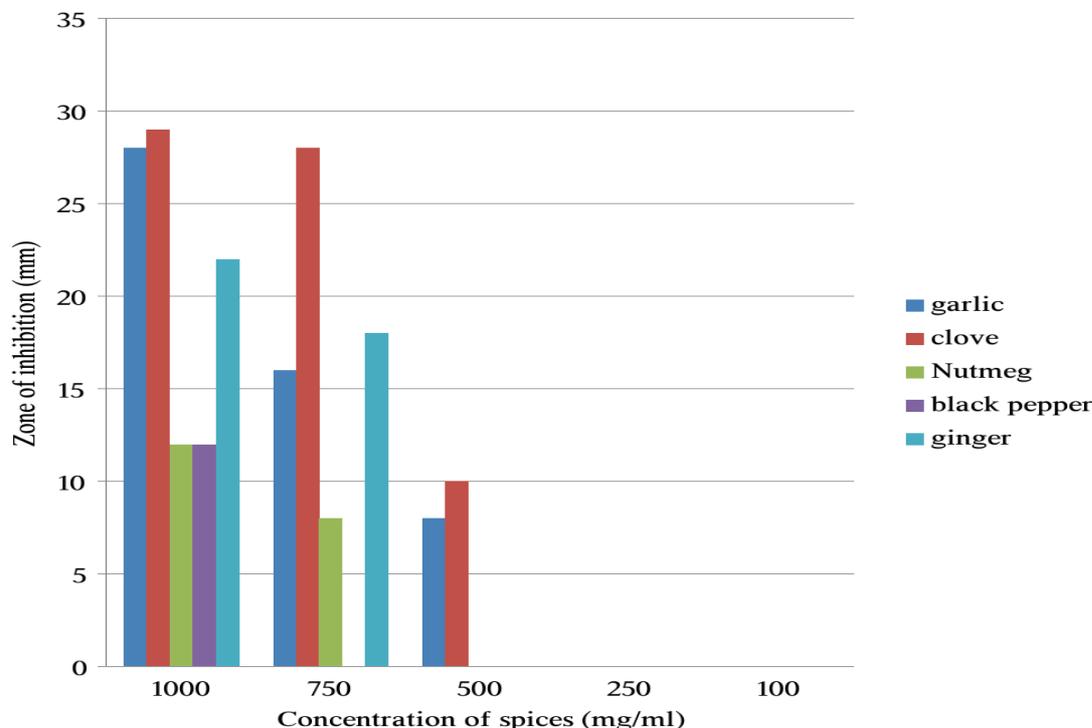


Fig. 3 Effect of aqueous spices extract on *staphylococcus aureus*

Figure 4 showed that clove had the highest zone of inhibition of 24mm in 1000mg and 20mm in 750mg. *Piper guineese* had the lowest zone of inhibition of 10mm at 1000mg and 8mm at 750mg. Also garlic

and ginger are active at 1000mg and 750mg only with zone of inhibition of 22mm and 20mm. Black pepper is only active at 1000mg with a zone of inhibition of about 12mm.

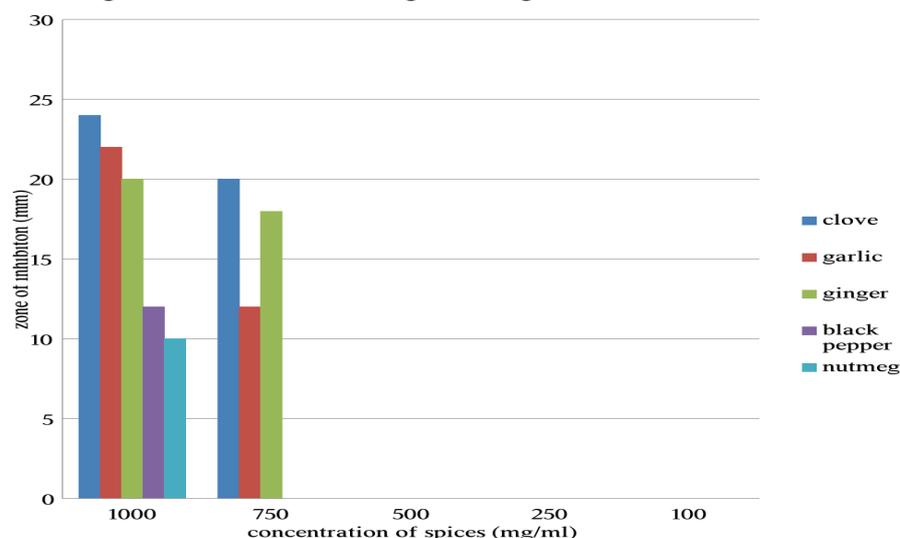


Fig. 4 Effect of aqueous spices extract on *Escherichia coli*.

Figure 5 Showed the comparative analysis of extract of some local spice and two antibiotics on *staphylococcus aureus*. The ethanol extracted spice had the highest effect on *staphylococcus aureus* compared to the aqueous extract and the used antibiotic. At 1000mg concentration clove

spice had 35mm zone of inhibition on *Staphylococcus aureus*, garlic had 34mm zone of inhibition at 1000mg concentration. While antibiotics nitrofurantoin had 26mm zone of inhibition on the same clinical isolate and tetracycline had 14mm zone of inhibition.

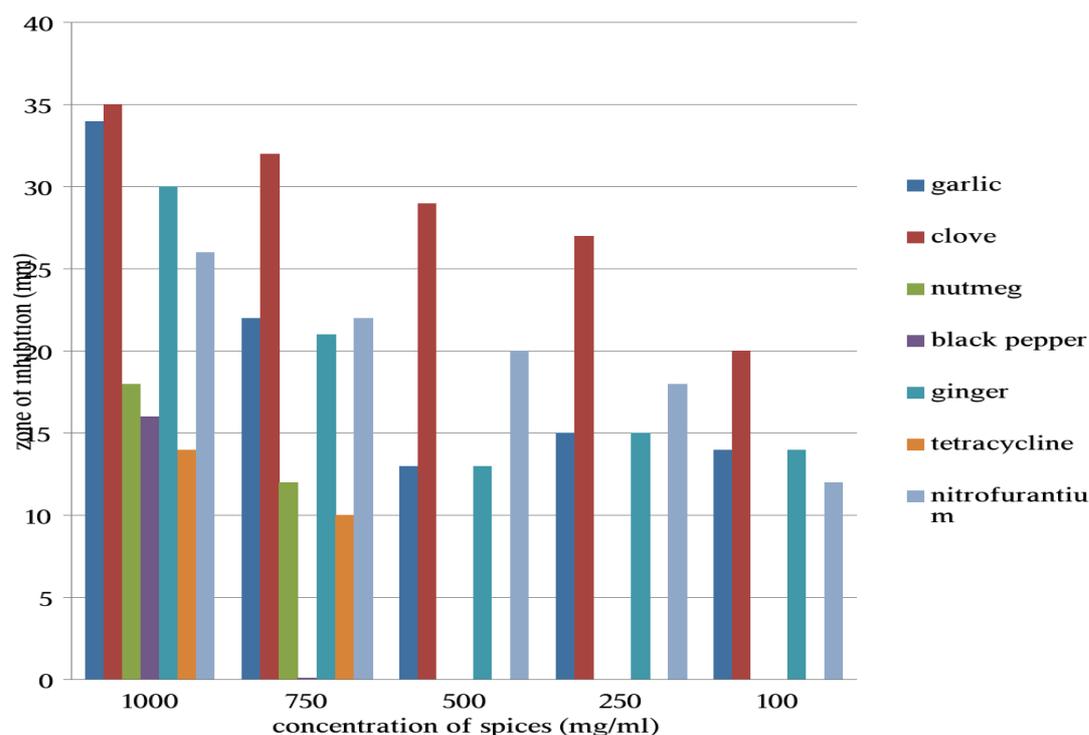


Fig.5 Comparative analysis of some local spices and antibiotics on *Staphylococcus aureus*

Figure 6 showed the comparative analysis of extract of some local spice and two antibiotics on *Escherichia coli*. The ethanol extracted spice showed the highest effect on *Escherichia coli* compared to the aqueous extract and the used antibiotic. At 1000mg concentration clove (spice) had

30mm zone of inhibition on *Escherichia coli* and garlic had 28mm zone of inhibition. While antibiotic ciprofloxacin had 28mm zone of inhibition on the same clinical isolate while chloraphenicol had 26mm zone of inhibition.

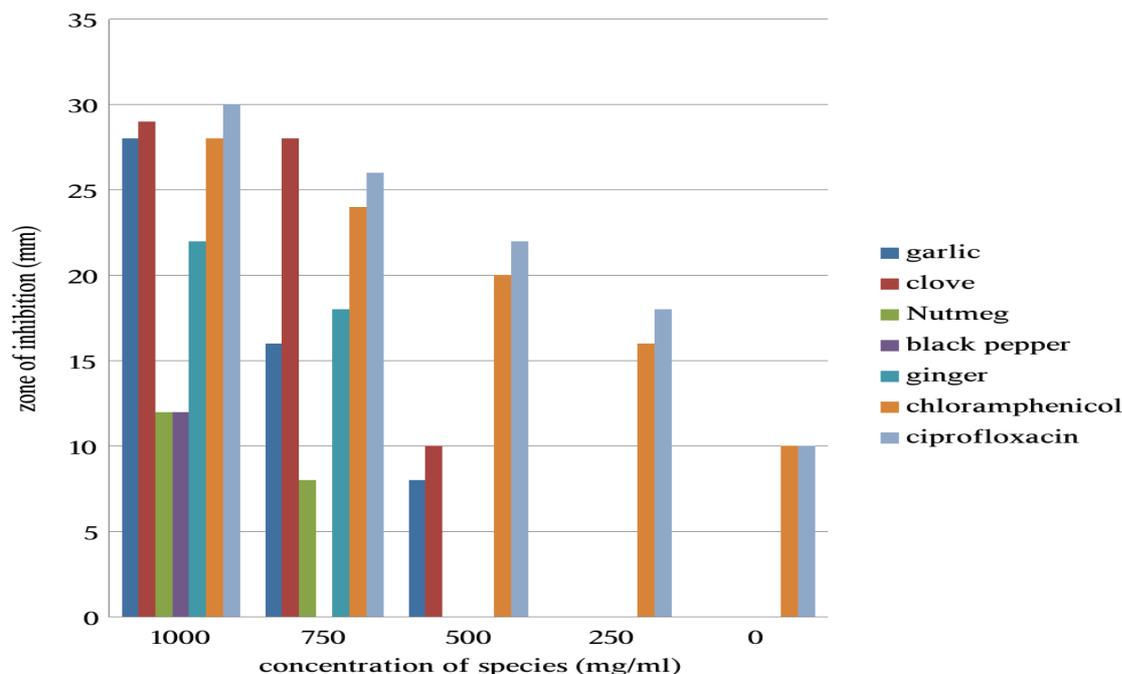


Fig. 6. Comparative analysis of some local spices and antibiotics on *Escherichia coli*

DISCUSSION

The results obtained showed that clove extract had the highest zone of inhibition. Garlic showed positive effect on the clinical isolate. Garlic had 34mm zone of inhibition at 1000mg concentration and ginger 30mm zone of inhibition. Nutmeg requires a very high concentration whereby the spice is only effective at 1000mg and 750mg with a zone of inhibition of 18mm and 12mm respectively, similar result was observed by (Busani *et al.*, 2006), this support the use of clove as food additives to control growth of food borne pathogen bacteria in food product. Also garlic extract showed a good antibacterial activity at all concentration too, (1000mg/100ml, 750, 500, 250 and 100) on the clinical isolates (*Staphylococcus aureus* and *Escherichia coli*). The zone of inhibition is 29mm. However, garlic extract was able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* at all concentration. The antibacterial activity of garlic is reported

to be due to the action of allicin or diallylthiosulfinate acid or diallyl disulphide (Huges and Lawson 1991).

In fig. 4, aqueous extract spices are active at high concentration and inactive at low concentration, thus, the study suggest that the inhibition of bacterial growth activity of aqueous extract is dose dependent. Clove showed the highest zone of inhibition of 24mm at 100% and 20mm zone of inhibition at 750 while nutmeg had the lowest zone of inhibition of 10mm at 1000 and 8mm at 750. Black pepper is only active at 1000mg with a zone of about 12mm. similar result was observed by (Okigbo and Igwe, 2007). Black pepper showed least zone of inhibition compared to the other four tested spices. At 1000mg concentration, moderate zone of inhibition of about 16mm was obtained on *Staphylococcus aureus* and it is ineffective at 500, 250 and 100 concentration. *Escherichia coli* is resistant to the extract of piper nigrum at all concentration.

This result is in agreement with (Okigbo and Ogbonanya, 2006; Amadioha and Obi, 1999). The ethanol extract of black pepper showed a concentration dependent gradient in the level of inhibition against the clinical isolate.

Comparative studies using antibiotic showed that clove, ciprofloxacin and garlic are active against the bacteria used in this study. Garlic, clove and ciprofloxacin had similar levels of antimicrobial activity against these isolates; this was attributed to similarity in the mechanism of action of garlic and ciprofloxacin. Allicin, the active ingredient of garlic (Hughes and Lawson, 1991) acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target (Feldberg *et al.*, 1988).

The comparative analysis of extract of some local spice and antibiotics on *staphylococcus aureus* and on *Escherichia coli* showed that ethanolic extracted spice have highest antibacterial effect on *staphylococcus aureus* and *Escherichia coli* compared to the aqueous extract and the antibiotics in figure 5 and 6 respectively.

At 1000 concentration clove spice had 35mm zone of inhibition on *staphylococcus aureus*, and garlic, 34mm. While antibiotic

nitrofurantoin had 26mm zone of inhibition on the same clinical isolate, *Staphylococcus aureus* and *Escherichia coli*. Tetracycline had the lowest zone of inhibition of 14mm. At the same 1000mg concentration, clove had 30mm zone of inhibition on *Escherichia coli*. Garlic had 28mm zone of inhibition and the antibiotic ciprofloxacin had 28mm zone of inhibition too on the same clinical isolate, chloramphenicol had 26mm zone of inhibition, similarly ciprofloxacin inhibits bacterial DNA gyrase and thus interferes with DNA transcription and other activities involving DNA similar result was observed by (Prescott *et al.*, 2005).

CONCLUSION

This study was done to compare the antibacterial activities of some local spices (clove, garlic, nutmeg, black pepper and ginger) on *Staphylococcus aureus* and *Escherichia coli*. The study supports their antimicrobial properties, when these extract are subjected to microorganisms. The study showed that some spices had the higher antibacterial activity compared to the standard antibiotics used, which has provided scientific justification for the medicinal use of the spices.

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