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Antimicrobial activities of aqueous and ethanolic extracts of some natural spices (garlic, turmeric, thyme and onions) on some clinical isolates

Nnenna Jennifer Omorodion * and Esther Chinwe Ujoh

Department of Microbiology, University of Port Harcourt, PMB 5323, Rivers State, Nigeria.

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Abstract

Different types of spices used daily have been documented to have antimicrobial and medicinal value as well. Most bacteria are sensitive to the extracts from plants such as, garlic, onion, thyme and turmeric etc. Spices such as these have been used as antimicrobial agents in their raw form for the treatment of wounds, injuries and joint pains etc. The present study was conducted to investigate the antibacterial activity of these natural spices. Different concentrations of extracts were prepared by using two solvents; water and ethanol, the test organisms used were *Escherichia coli* and *Staphylococcus aureus*. All the spices used were effective against the test organisms, but the best activity was shown in garlic, while the least activity was shown in thyme, in general, the ethanolic extracts were more reactive than the aqueous extracts. Natural spices could be a potential source for antimicrobial agents. Validation of natural spices as alternative and complementary medicine can be achieved by several in vivo trials through which the safe use of such products can be assured. This study emphasizes the therapeutic potential of spices and the need to enhance its exploitation on this regard.

Keywords: Spices; Antimicrobial agents; Concentrations; *Escherichia coli*; *Staphylococcus aureus*

1. Introduction

Spices are commonly used for flavoring foods since the ancient times, and as natural medicines against different of human diseases. Spices could be a seed, fruit, root, bark, berry, bud or vegetable substance that is primarily used as natural colorants [Ravindran *et al.*, 2006], flavors, antioxidants [Shobana and Akhilender, 2000] and antimicrobials [Ceylan and Fung, 2004]. The use of spices corresponds mainly with the food industry, but they are also used in medicine [Shan *et al.*, 2007], cosmetics, perfumery and pharmaceuticals industry [Peter and Shylaja, 2012]. In medicine, spices are used for different applications, such as stimulants, diuretics, carminatives, anti-inflammatory, stomachic, antibiotics, digestives, astringents, anthelmintics, expectorants and tonics etc [Chattopadhyay *et al.*, 2004]; [Platel and Srinivasan, 2004]

Garlic belongs to the genus *Allium* and is closely related to the onion, rakkoyo (an onion found in Asia), scallion, chire, leek and shallot. Garlic has been used all over the world for thousands of years. Records indicate that garlic was in use when the Giza pyramids were built, about 5,000 years ago. Richard S. Rivlin wrote in the 'Journal of nutrition' that the ancient Greek physician Hippocrates (circa. 460-370BC), known today as 'the father of western medicine', prescribed garlic for a wide range of conditions and illnesses. Hippocrates promoted the use of garlic for treating respiratory problems, parasites, poor digestion, and fatigue. Currently garlic is widely used for several conditions linked to the blood system and heart, including atherosclerosis (hardening of the arteries), high cholesterol, heart attack, coronary heart disease, and prevention of lung cancer, prostate cancer, breast cancer etc. [Benkeblia, 2004] [Azene, and Worku, 2015]

* Corresponding author: Omorodion Nnenna JP
Department of Microbiology, University of Port Harcourt, PMB 5323, Rivers State, Nigeria.

TUMERIC (*curcuma longa*), is a bright yellow spice used throughout Asia for centuries, it has in recent decades been embraced by the west, not just for its ability to satisfy our appetite for curry, but for its impressive list of health benefits. Turmeric is quite literally, hot stuff. It is grown for its root, it has an ancient history of uses in cooking, fabric dyeing, cosmetics and traditional medicine in China and India. Its potent ingredient, curcumin, not only gives turmeric its golden colour, but also has a dazzling array of properties that are beneficial to health. Long term inflammation has been implicated in most chronic conditions such as heart disease and cancer so, must be controlled. The curcumin in turmeric has proven, strong anti-inflammatory that block bowel diseases, amongst others. Turmeric is also a powerful antioxidant as curcumin has been shown to be a robust scavenger of oxygen free radicals, which are chemically active molecules that cause damage to the body cells, it can play a part in preventing and managing heart disease. [Melvin *et al.*, 2009 [Uma 2012]

ONIONS belong to the Allium family of plants, which also includes chives, garlic and leeks. They have characteristic pungent flavours and some medical properties. Onions vary in shape, size, colour and flavor. The most common types are red, yellow and white onions, their task can range from sweet and juicy to sharp, spicy and pungent, often depending on the season in which people grow and consume them according to the food and agriculture organism of the United Nations, China is the biggest producer of onions worldwide. It is common knowledge that chopping onions causes watery eyes. However, onions may also provide health benefits. These may include; reducing the risk of several types of cancer, improving mood, and maintaining skin and good health. Researchers have found that the risk of colorectal cancer was 79% in those who regularly consumed allium vegetables, such as onions, some experts also hypothesize that onions inhibit tumor growth and cell mutation. One cup of chopped onions also provides at least 13.11% of an adults recommended daily intake of vitamin C as an antioxidant, as a good source of vitamin C, onions may support the building and maintenance of collagen, which provides structure to skin and hair. [Brewster1994].

THYME is a Mediterranean herb with dietary, medicinal and ornamental uses. The flowers, leaves and oil of thyme have been used to treat a range of symptoms and complains which include; diarrhea, stomach ache, arthritis, sore throat etc, the most common variety is *thymus vulgaris*, it is one of a naturally occurring class of compounds known as biocides. These are substances that can destroy harmful organisms such as infectious bacteria. Thyme has strong antimicrobial properties, a study from year 2010 suggest that thymol can reduce bacteria resistance to common drugs, including penicillin. A polish study tested thyme oil and lavender oil and they observed that thyme oil was effective against resistant strains of *Staphylococcus*, *Enterococcus*, and *Escherichia* and *Pseudomonas* bacteria. The antibacterial activity of essential oils of thyme, marjoram, chamomile was investigated against *Bacillus cereus* and *Bacillus subtilis* by [Gurgulova *et al.* 2006] and [Abd El Mageid *et al.* 2009] and they reported that the highest antibacterial activity recorded for the essential oils of thyme and wild marjoram for all strains and the oil of chamomile had low antibacterial activity against the same strains. Also, [Witkowska *et al.* 2013] indicate that crude extracts of some herbs and spices possess *in vitro* activity against food spoilage and pathogenic bacteria, displayed bactericidal or bacteriostatic activities with consequent damage to bacterial cell membranes of both Gram positive and Gram-negative bacteria.

The uses of natural herbs/spices such as turmeric, garlic, etc. have become more common in not just rural areas but also the urban areas. Most of these spices/herbs e.g. garlic act as potential inhibitor for food pathogens. The problem of drug resistance is presently a global issue as a result of continuous wrong use of antibiotics. If these natural agents are found to be effective against common pathogens, it then follows that they can be used in place of base line antibiotics as remedy, again these natural spices/herbs are also food condiments, using them will eliminate side effects, that is normally found in commercial antibiotics [Melvin *et al.*, 2009]. The aim of this study is to determine the effect of antimicrobial activities of aqueous and ethanolic extracts of garlic, turmeric, thyme and onions have on some clinical isolates.

2. Material and methods

Microorganisms Gram negative bacteria; *Escherichia coli*, Gram positive; *Staphylococcus aureus* from different sources {urine, swab and aspirate samples} were collected from University of Port Harcourt teaching hospital, Port Harcourt City (UPTH) and used for the antimicrobial studies. All the isolates were maintained on nutrient agar slants at 4°C.

2.1. Plant Collection and Preparation

Fresh bulbs and leaves of garlic, turmeric, onions and dried thyme leaves were collected from the vegetable garden in Port Harcourt.

2.2. Preparation of aqueous and ethanolic extracts

The papery and epidermal skin of the garlic and onions were peeled with the turmeric, then they were washed with distilled water thoroughly and cut longitudinally into smaller pieces to increase the surface area for quicker drying. The cut slices were dried in an oven at about 60 °C. The result was dry and brittle cloves with a brownish colouration. The properly dried spices were ground into fine powder using a mechanical grinder and stored in an air tight container to prevent moisture penetration.

The Soxhlet extraction process was used for the ethanolic extraction of garlic, thyme, turmeric and onions. The ground plant material was placed in the extraction chamber which was suspended above the flask containing ethanol below a condenser. The flask was heated and solvent and the solvent evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level, it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the ethanol was removed and concentrated on a water bath for 78 °C and then left to dry at room temperature as described by [Mishra and Behal, 2010].

For the aqueous extraction, the ground plant samples were each extracted with distilled water by maceration. 80g of each of the sample was soaked with 200 ml of distilled water for 72 hours at room temperature. After that the resulting extracts were filtered using filter paper. The filtrates obtained were concentrated by heating over water bath. The extracts were then put into labelled containers. [Mishra and Behal 2010].

2.3. Maintenance of bacterial culture and inoculum preparation

Pure cultures were refreshed and maintained on nutrient agar slants and plates on regular basis. The cultures were streaked on sterile nutrient agar plates and kept in incubator for 24 hours at 37°C. Bacterial cultures were refreshed after every 3 to 4 days to avoid contamination. Inoculum was prepared by growing the pure bacterial culture in nutrient broth over night at 37 °C.

2.4. Antimicrobial Sensitivity Testing Using Disc Diffusion Method.

Filter paper disc of 5mm diameter using Whatman no. 1 filter paper was prepared and sterilized. The test microorganisms were transferred from nutrient broth to sterile Muller Hinton agar plates with the help of sterile cotton swabs. Using an ethanol dipped and flamed forceps the discs were aseptically placed over the Muller Hinton agar plates seeded with the test microorganisms. 10-µL of the various spice extract, ethanol extract and aqueous extract were aseptically transferred to each disc at all dilutions that were made in triplicate. Plates were incubated in an upright position at 37 °C for 24 hours. 10 µL of 95% ethanol was added in sterile filter paper disc as negative control. Triplicate sample of each dilution was tested. After 24hours the diameter of zone of inhibition were measured in mm and results were recorded. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and >9mm zone was considered as inactive; 9-12 mm as partially active; while 13-18 mm as active [Junior and Zani, 2000]. The mean and standard deviation of the diameter of inhibition zones were calculated.

2.5. Antimicrobial Sensitivity Testing

The cultures were enriched in nutrient broth for 6-8hrs at 37°C using a sterile cotton swab; the cultures were aseptically swabbed on Muller Hilton agar and allowed to dry for 5mins then, an ethanol dipped and flamed forceps was used to pick the antibiotic disc which was aseptically placed on the seeded surface of the Muller Hilton agar sufficiently separated in other to avoid over lapping. The susceptibility of the bacteria strains to various antibiotics was performed by the following National Committee for Clinical laboratory Standard Recommendation. The zones of inhibition were recorded after incubating at 37°C for 24 hrs. The test microorganisms i.e *Escherichia coli* and *Staphylococcus aureus* were also tested for their sensitivity against the antibiotics gram positive organism Cloxacillin (5 µg) Gentamycin (10 µg) Cotrimoxazole (25 µg) Chloramphenicol (10 µg) Augmentin (30µg) Amoxicillin (30 µg) AND Gram negative organism Nitrofuratoin (30µg) Gentamycin (10 µg) Naldixic (30 µg) Ofloxacin (30 µg) Augmentin (30 µg) Tetracycline (10 µg) Amoxicillin (30 µg) Cotrimoxazole (25 µg] by disc diffusion method.

2.6. Statistical analysis

All the values of inhibition zone were analyzed using Analyses of Variance (ANOVA). Differences between groups were considered significant at P < 0.05 level of significance.

3. Results

DIAMETRE OF ZONES OF INHIBITION						
BY TEST ORGANISMS, CONCENTRATIONS, ANTIMICROBIAL AGENTS						
TEST ORGANISMS	10.00%	12.50%	16.70%	25.00%	50.00%	Total
STAPH	4	18	28	53	76	179
THYME AQUEOUS EXTRACTS	0	0	0	0	0	0
TUMERIC AQUEOUS EXTRACTS	0	0	0	0	0	0
TUMERIC ETHANOIC EXTRACTS	0	0	0	3	6	9
THYME ETHANOIC EXTRACTS	0	0	0	4	8	12
ONION ETHANOIC EXTRACTS	0	0	4	8	12	24
ONION AQUEOUS EXTRACTS	0	0	2	10	15	27
GARLIC AQUEOUS EXTRACTS	4	10	10	12	15	51
GARLIC ETHANOIC EXTRACTS	0	8	12	16	20	56
E. COLI	8	25	37	77	113	260
THYME AQUEOUS EXTRACTS	0	0	0	0	0	0
TUMERIC AQUEOUS EXTRACTS	0	0	0	0	4	4
THYME ETHANOIC EXTRACTS	0	0	0	6	10	16
TUMERIC ETHANOIC EXTRACTS	0	0	0	10	14	24
ONION AQUEOUS EXTRACTS	0	0	4	10	20	34
ONION ETHANOIC EXTRACTS	0	4	6	14	22	46
GARLIC ETHANOIC EXTRACTS	0	10	14	19	23	66
GARLIC AQUEOUS EXTRACTS	8	11	13	18	20	70
Total	12	43	65	130	189	439

Figure 1 Summary of the total activity of the antimicrobial effect of the spices used in this study. Highlighting the resistant (red columns) and the very sensitive ones (blue columns) according to their concentrations. Appendix I - Appendix VIII

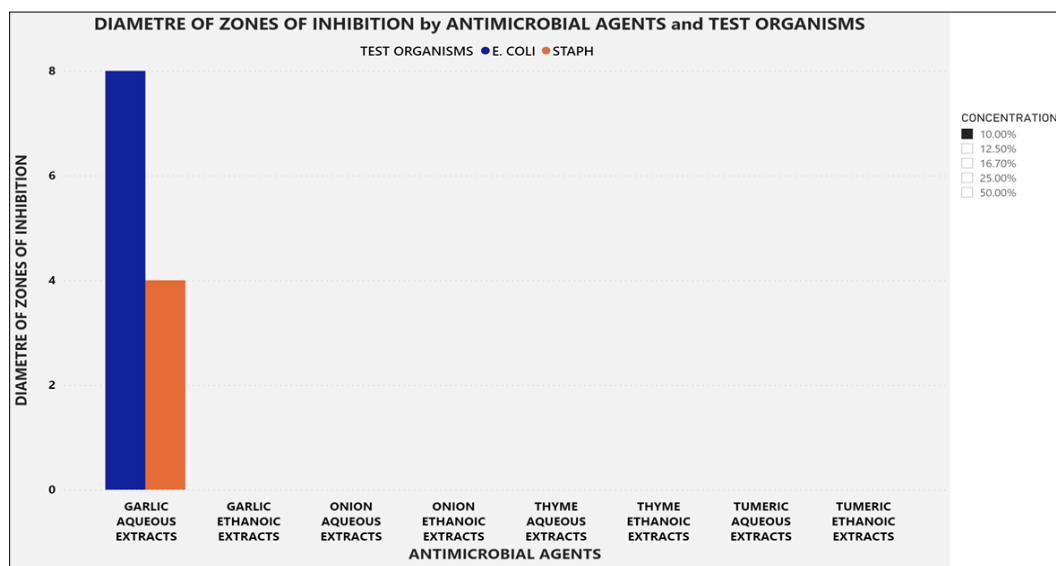


Figure 2 The zones of inhibitions obtained using the various antimicrobial agents at 10% concentration

Table 1 Antibiotics Sensitivity of Gram-Positive bacteria (*Staphylococcus aureus*) in (mm)

Antibiotics	CXC	GEN	COT	AUG	CHL	AMY	ERY	TET
<i>Staph aureus</i>	14	9	16	26	22	17	15	11

Key: Cloxacillin (5µg) Gentamycin (10µg) Cotrimoxazole (25µg) Chloramphenicol (10µg) Augmentin (30µg) Amoxicillin (30µg) Erythromycin (5µg) Tetracycline (10µg)

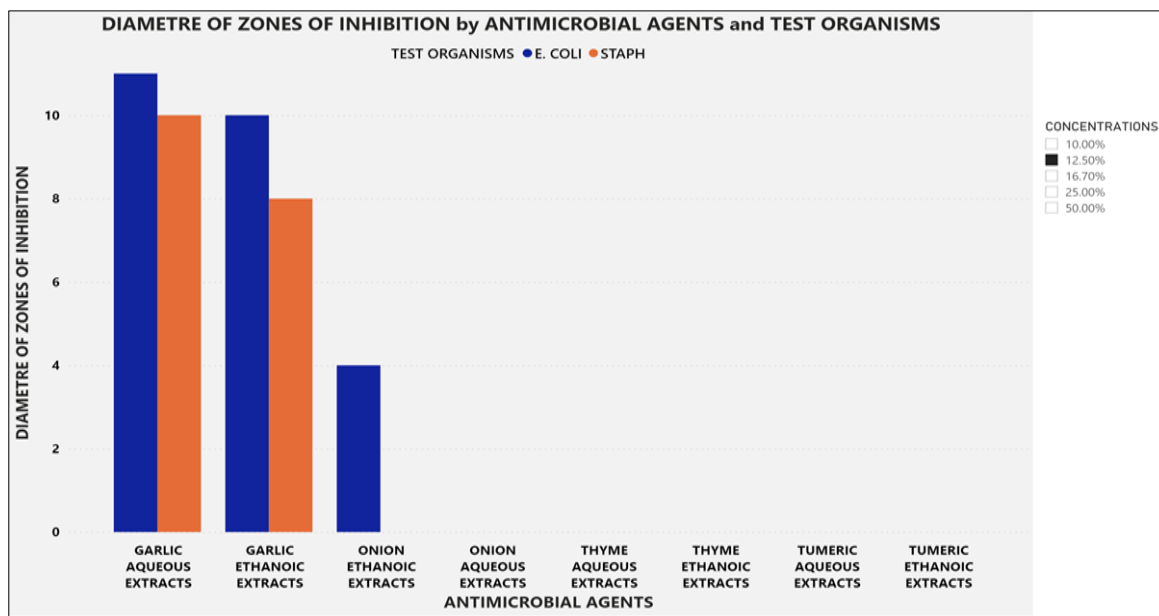


Figure 3 The zones of inhibition using the various antimicrobial agents at 12.5% concentration

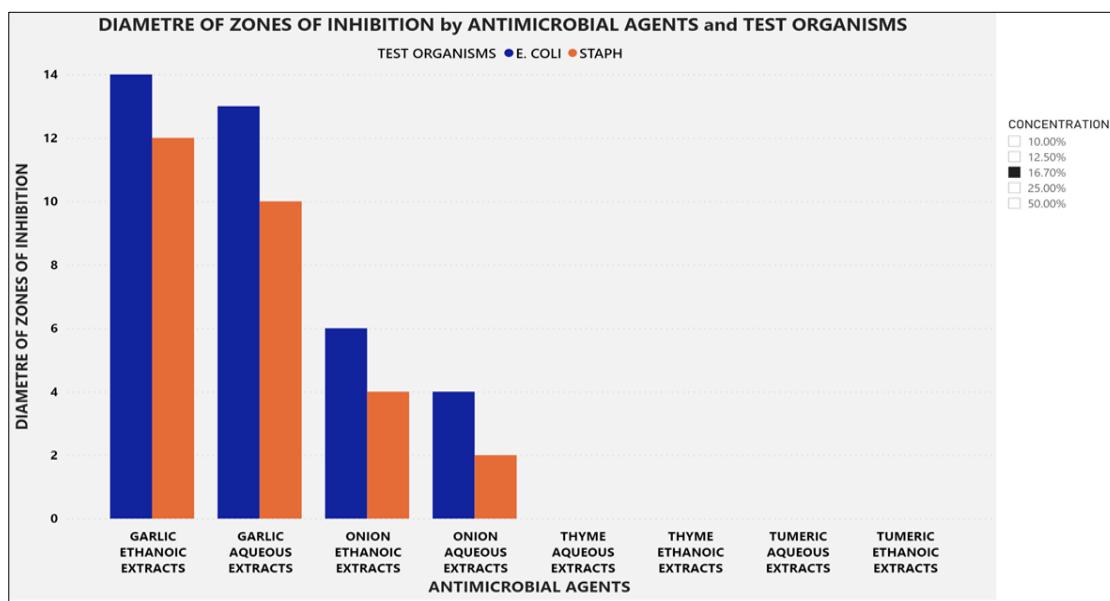


Figure 4 The zones of inhibition obtained using the various antimicrobial agents at 16.7% concentration

The spices; garlic (*Allium sativum*), turmeric (*Curcuma longa*), onion (*Allium cepa*) and thyme (*Thymus vulgaris*) were tested against *Escherichia coli* and *Staphylococcus aureus*. Most of the spices used were effective against the test organisms, however the best activities were shown by garlic giving a maximum zone of inhibition of 23mm for *E. coli* and 20mm for *Staph aureus*. The ethanolic extracts of the garlic, turmeric, onion and thyme gave wider zones of inhibition compared to their aqueous extracts showing that the ethanolic extracts were more sensitive on the microorganisms than the aqueous extracts. Onion formed a maximum zone of inhibition of 22mm on *E. coli* and 12mm on *Staph aureus*. Turmeric formed a zone of inhibition of 14mm on *E. coli* and 6mm on *Staphylococcus aureus*. Thyme formed a zone of inhibition of 10mm on *E. coli* and 8mm on *Staphylococcus aureus* as shown in fig 2- 6

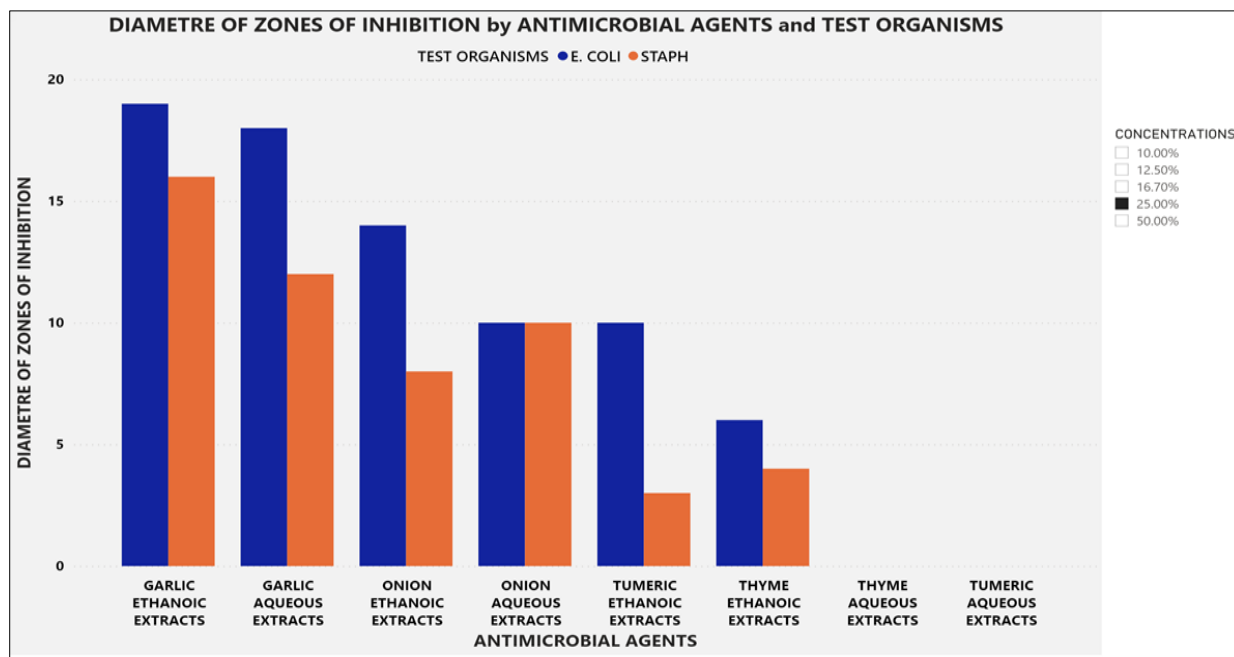


Figure 5 The zones of inhibition obtained using the various antimicrobial agents at 25% concentration

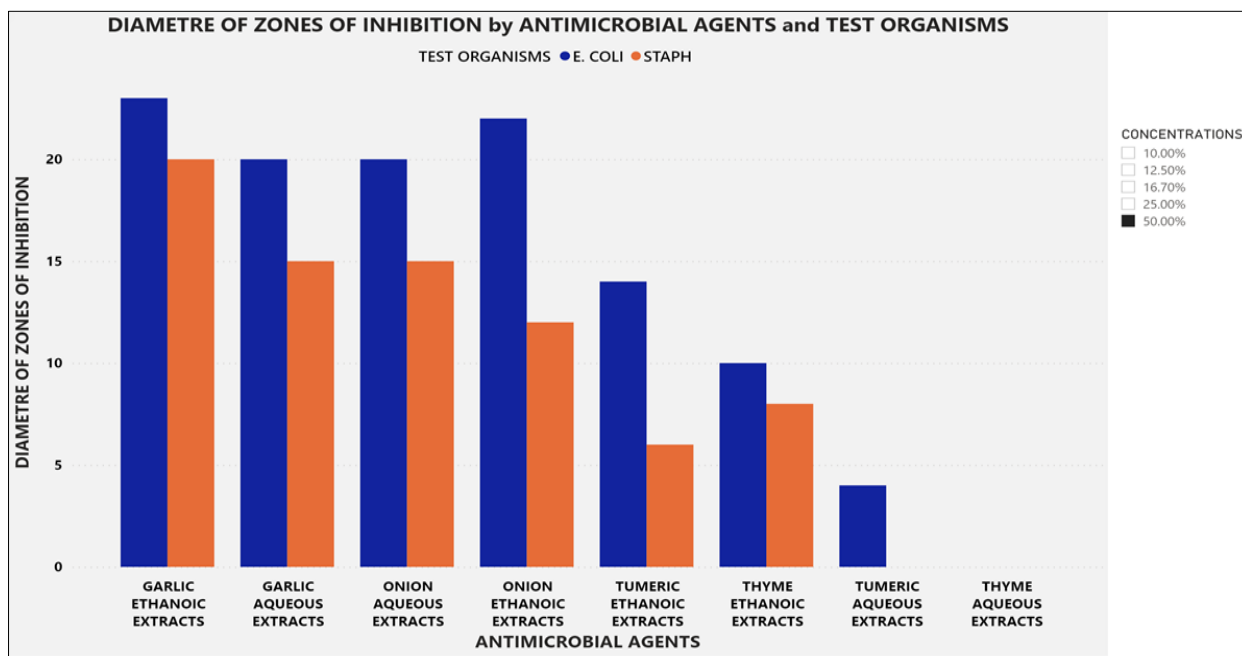


Figure 6 The zones of inhibition obtained using the various antimicrobial agents at 50% concentration

Table 2 Antibiotics Sensitivity of Gram-negative bacteria (*Escherichia coli*) in (mm)

Antibiotics	NAL	GEN	NIT	OFL	AUG	TET	AMX	COT
<i>E coli</i>	-	21	28	35	-	-	-	22

Key: Nitrofuratoin (30µg) Gentamycin (10µg) Naldixic (30µg) Ofloxacin (30µg) Augmentin (30µg) Tetracycline (10µg) Amoxicillin (30µg) Cotrimoxazole (25µg)

4. Discussion

Spices have since ancient times been an important group of agricultural commodities being used by many civilizations all over the World adding flavor and nutritional values to a wide variety of food. However, such spices are no longer used for only their culinary effects. The present study was aimed at investigating the potential of extensively used culinary spices commonly known as turmeric, garlic, thyme and onion as effective antimicrobial agents that could possibly contribute to the “natural antibiotic system. Two clinical bacterial isolates microbial species namely: *Staphylococcus aureus*, *Escherichia coli* were used. Among all the spices, garlic showed the best activity at all concentration both in aqueous and ethanol solvents. This is in agreement with the work done by [Sana Mukhtar and Ifra Ghorri 2012] [Safithri, et al., 2011]. Therefore, it could be used to cure diseases associated with *Escherichia coli* and *Staphylococcus aureus*, or used to develop drugs associated with the said organisms. Thyme on the other hand showed no form of activity at all in majority of the concentrations. Garlic was observed to be more sensitive on *Escherichia coli* as compared to *Staphylococcus aureus*, which could be as a result of the resistant capabilities in *Staphylococcus aureus*. However, ethanolic extracts of garlic was more effective than the aqueous extract. The same could be seen in the other spices, as their ethanolic extracts were more effective compared to their aqueous extracts. Between the two tests organisms, maximum activity was shown against *Escherichia coli* the serotype of *E. coli* used in the study may have influence this activity. [Chandara *et al.* 2005] reported that turmeric is effective against *E. coli*, and *B. subtilis*, which is a gram-positive organism due to the presence of a phenolic compound, curcuminoid. The presence of essential oil, an alkaloid, curcumins and turmerol and valeric acid are responsible for antibacterial action of spices which involve the hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, membrane disruption and destruction of electron transport system and cell wall disruption. According to [Tijjani. *et al.*, 2017], an antimicrobial agent which the diameter of the zone of inhibition is above 3 mm, the organism is said to be sensitive but if it is 2 mm or less than that, the organism is said to have resistance over the particular agent.

Therefore considering the statement of Tijjani *et al.*, and comparing the result obtained in in this study , shows that the ethanolic extracts of the spices have antimicrobial effect in at least two of the concentrations (50% and 25%), the aqueous extracts of thyme having zero zone of inhibition in all the concentrations have no antimicrobial effect, the aqueous extract of turmeric with 4 mm diameter zone of inhibition will have antimicrobial effect on *Escherichia coli* at 50% concentration, the aqueous extracts of onions have antimicrobial effect on both *Escherichia coli* and *Staphylococcus aureus*, at 16.7, 25 and 50% concentrations, the aqueous extracts of garlic have antimicrobial effect on *Escherichia coli* and *Staphylococcus aureus*, in the five different concentrations.

From this study it is deduced that the spices; garlic, onion at a 50% concentration could be used for therapeutic studies. Among all the spices used garlic was most effective, so it can be used to develop new antimicrobials. The antimicrobial activity of aqueous extracts could be due to anionic components such as thiocyanate, nitrate, chlorides and sulphates in addition to many other compounds naturally present in plants. [Darout, 2000]. The ethanolic extracts showed better results as compared to aqueous as being organic dissolves more organic compounds resulting in the release of greater amount of active antimicrobial components [Sana Mukhtar and Ifra Ghorri, 2012]. The mean difference between the spices used were significant on *Escherichia coli* and *Staphylococcus aureus*.

S. aureus was highly susceptible to Augmentin and Chloramphenicol with 26mm and 22mm zones of inhibition while resistant to Gentamicin and tetracycline. This conforms to the findings of [Aboaba and Efuwape, 2001] [Bankole *et al.*, [2019] who reported similar result. *E. coli* was sensitive to Ofloxacin and Nitrofuratoin this conforms to the study of [Aboaba and Efuwape, 2001] [Bankole *et al.*, 2019]

The result of the study highlights the usefulness of spices in the treatment of diseases and the need to enhance its exploitation on this regard. This is particularly of urgent interest when the growth rate of multi-resistant drug strains of bacteria worldwide is considered as a threat. The study also showed that spices extracts possess difference antibacterial activities, most spices only serve as nutritive or nutrient supplement, as flavours, aroma, but rarely serve as antibiotics.

Appendix**Appendix I** Antimicrobial activity of aqueous extracts of garlic on selected organisms

Test organisms	Concentrations	Diameter of zones of inhibition (mm)
<i>E. coli</i>	50%	20
<i>Staph. aureus</i>	50%	15
<i>E. coli</i>	25%	18
<i>Staph. aureus</i>	25%	12
<i>E. coli</i>	17%	13
<i>Staph. aureus</i>	17%	10
<i>E. coli</i>	13%	11
<i>Staph. aureus</i>	13%	10
<i>E. coli</i>	10%	8
<i>Staph. aureus</i>	10%	4

Appendix II Antimicrobial activity of aqueous extracts of tumeric on selected organisms

Test organisms	Concentrations	Diameter of zones of inhibition (mm)
<i>E. coli</i>	50%	4
<i>Staph. aureus</i>	50%	0
<i>E. coli</i>	25%	0
<i>Staph. aureus</i>	25%	0
<i>E. coli</i>	17%	0
<i>Staph. aureus</i>	17%	0
<i>E. coli</i>	13%	0
<i>Staph. aureus</i>	13%	0
<i>E. coli</i>	10%	0
<i>Staph. aureus</i>	10%	0

Appendix III Antimicrobial Activity of Aqueous Extracts Of Onion on Selected Organisms

Test organisms	Concentrations	Diameter of zones of inhibition (mm)
<i>E. coli</i>	50%	20
<i>Staph. aureus</i>	50%	15
<i>E. coli</i>	25%	10
<i>Staph. aureus</i>	25%	10
<i>E. coli</i>	17%	4
<i>Staph. aureus</i>	17%	2
<i>E. coli</i>	13%	0
<i>Staph. aureus</i>	13%	0
<i>E. coli</i>	10%	0
<i>Staph. aureus</i>	10%	0

Appendix IV Antimicrobial Activity of Aqueous Extracts Of Thyme on Selected Organisms

Test organisms	Concentrations	Diameter of zones of inhibition (mm)
<i>E. coli</i>	50%	0
<i>Staph. aureus</i>	50%	0
<i>E. coli</i>	25%	0
<i>Staph. aureus</i>	25%	0
<i>E. coli</i>	17%	0
<i>Staph. aureus</i>	17%	0
<i>E. coli</i>	13%	0
<i>Staph. aureus</i>	13%	0
<i>E. coli</i>	10%	0
<i>Staph. aureus</i>	10%	0

Appendix V Antimicrobial Activity of Ethanolic Extracts Of Garlic on Selected Organisms

Test organisms	Concentrations	Diameter of zones of inhibition (mm)
<i>E. coli</i>	50%	23
<i>Staph. aureus</i>	50%	20
<i>E. coli</i>	25%	19
<i>Staph. aureus</i>	25%	16
<i>E. coli</i>	17%	14
<i>Staph. aureus</i>	17%	12
<i>E. coli</i>	13%	10
<i>Staph. aureus</i>	13%	8
<i>E. coli</i>	10%	0
<i>Staph. aureus</i>	10%	0

Appendix VI Antimicrobial activity of ethanolic extracts of onion on selected organisms

Test organisms	Concentrations	Diameter of zones of inhibition (mm)
<i>E. coli</i>	50%	22
<i>Staph. aureus</i>	50%	12
<i>E. coli</i>	25%	14
<i>Staph. aureus</i>	25%	8
<i>E. coli</i>	17%	6
<i>Staph. aureus</i>	17%	4
<i>E. coli</i>	13%	4
<i>Staph. aureus</i>	13%	0
<i>E. coli</i>	10%	0
<i>Staph. aureus</i>	10%	0

Appendix VII Antimicrobial activity of ethanolic extracts of tumeric on selected organisms

Test organisms	Concentrations	Diameter of zones of inhibition (mm)
<i>E. coli</i>	50%	14
<i>Staph. aureus</i>	50%	6
<i>E. coli</i>	25%	10
<i>Staph. aureus</i>	25%	3
<i>E. coli</i>	17%	0
<i>Staph. aureus</i>	17%	0
<i>E. coli</i>	13%	0
<i>Staph. aureus</i>	13%	0
<i>E. coli</i>	10%	0
<i>Staph. aureus</i>	10%	0

Appendix VIII Antimicrobial activity of ethanolic extracts of thyme on selected organisms

Test organisms	Concentrations	Diameter of zones of inhibition (mm)
<i>E. coli</i>	50%	10
<i>Staph. aureus</i>	50%	8
<i>E. coli</i>	25%	6
<i>Staph. aureus</i>	25%	4
<i>E. coli</i>	17%	0
<i>Staph. aureus</i>	17%	0
<i>E. coli</i>	13%	0
<i>Staph. aureus</i>	13%	0
<i>E. coli</i>	10%	0
<i>Staph. aureus</i>	10%	0

5. Conclusion

The study has provided the justification for therapeutic potential of spices. The practice of using spices as supplementary or alternative medicine in developing countries like Nigeria will not reduce only the clinical burden of drug resistance development but also the side effects and cost of the treatment with allopathic medicine

Compliance with ethical standards*Acknowledgments*

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Disclosure of conflict of interest

Authors have declared that no competing interests exist

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