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ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOL EXTRACT OF TEN DIFFERENT COMMERCIAL SPICES AND HERBS AGAINST THREE CLINICAL BACTERIAL ISOLATES

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ABSTRACT

The use of spices in the treatment of diseases has not been fully exploited. Ethanol and aqueous extract of the following spices: Garlic (*Alium sativum*), Onion (*Allium cepa*), Cinnamon (*Cinnamomum verum*), Thyme (*Thymus vulgaries*), Ginger (*Zingiber officinale*), Lime leaf (*Citrus aurantifolia*), Curry (*Murraya koenigii*), Bay leaf (*Laurus nobilis*), Red pepper (*Capsicum annum* L.) and Tumeric (*Curcuma longa*) were tested for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae,* using agar well diffusion method at concentrations of 100, 75, 50 and 25mg/mL. The Minimum Inhibitory Concentration (MIC), synergistic effect of two spices in the same family and antimicrobial sensitivity were also determined. Aqueous and ethanol extract of cinnamon were the most effective against the test organism with zones of inhibition between 9mm to 25mm; closely followed by tumeric which also showed good inhibitory effect on the test organisms except that the aqueous and ethanol extract did not have any effect on *E. coli* and *S. aureus* respectively. Garlic–onion aqueous extract inhibited the test organisms with zones of inhibition ranging between 9mm to 24mm. Ginger - tumeric aqueous extract did not have any effect on *E. coli* and *S. aureus*. The MIC for aqueous and ethanol extract of cinnamon was 3.125mg/ml and 12.5mg/ml respectively. *E. coli* was highly susceptible to gentamicin than the remaining two organisms. This work has shown that both cinnamon and turmeric can be used as antimicrobial agents in the treatment of some diseases.

Keywords: ethanol, aqueous, spices, *K. pneumoniae, E. coli, S. aureus*

Acepted date: 24 October 2017

INTRODUCTION

Spices can be defined as 'vegetable products used for flavouring, seasoning and imparting aroma in foods'. Herbs are leafy spices, and some, can provide both spice seeds and leafy herbs (Douglas *et al.*, 2005). Herbs and spices are essential part of man's nutrition. They have been used to enrich the taste, colour and smell of food (Chandarana *et al.*, 2005); as traditional medicine for thousands of years (Babuskin *et al*., 2014); and also as food preservatives (Nakatani, 1994). They are used to a large extent in many Asian, African and other countries. Currently, in regard of their useful effects, the consumption of spices/herbs has been steadily improving in developed countries too (Indu *et al.,* 2006).

Phytochemicals are compounds responsible for the exceptional taste and smell in spices and herbs (Avato *et al.,* 2000). Some spices are known to kill (bactericidal) or stop (bacteriostatic) microbial growth (Ağaoğlu *et al.,* 2007). There has been an incredible upsurge in resistance to antibiotics by various bacterial pathogens (Wood *et al.,* 1996), which has led to the search by scientists for alternative antimicrobials like spices instead of other chemotherapeutic agents. *S. aureus*, *E. coli* and *K. pneumoniae* are all important human pathogens and are known to be causative agent of different diseases ranging from boil, pneumonia, meningitis to UTI (Prescott *et al*., 2008). These pathogens have been found by different investigators to be inhibited by at least one of the following: cinnamon, ginger, garlic, curry and lime leaf (Ağaoğlu *et al*., 2007; Ojo *et al*., 2007; Akintobi *et al*., 2013; Khan Pathan *et al.,* 2012; Malwal and Sarin, 2011).

Review of available literatures has showed that most research on antimicrobial properties of spices in Nigeria have been on ginger and garlic. Therefore, this research aimed at: extracting ten different spices using ethanol and water and determine their antibacterial activity and Minimum Inhibitory Concentration (MIC); also the synergistic effect of the spices in the same family was determined.

Materials and Methods

Samples collection

Ten (10) different commercial spices namely: garlic, onion, thyme, ginger, lime leaves, curry, bay leaf and red pepper were purchased from the major market in Ago-Iwoye, Ogun State while cinnamon and turmeric were purchased from Shoprite at Ibadan, Oyo State both in Nigeria.

Bacterial Isolates

The three test organisms namely: *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* used in this study were collected from the Federal Medical Centre, Abeokuta, Ogun State. They were further confirmed with biochemical tests such as glucose fermentation, catalase, oxidase, coagulase, urease and indole as described by Cowan and Steel (1985). The bacteria were maintained on nutrient agar slants at 4°C in the refrigerator.

Extraction of Spices

This was carried out according to the method of Joe *et al*. (2009) with little modification. The spices were dried in hot air oven at 35-40°C for 3 days and were ground using a sterile blender. One hundred (100) gram of the spices were weighed and soaked separately in ethanol and water for 3 days. The extracts were filtered through Whatman filter paper no.1. The filtrates were concentrated on a water bath. The extract was taken as 100%. The respective solvents were added to make concentrations of 25, 50, and 75mg/mL of the extracts. The extracts were used for further studies such as antibacterial activity and minimum inhibitory concentration (MIC)

Inoculum preparation

Colonies of the test organism were transferred into the test tube containing 5ml of sterile nutrient broth and standardized to 0.5McFarland turbidity.

Evaluation of Antibacterial Activity of the Spice Extracts

The spice extracts were screened for their antibacterial activity against the test organisms by agar well diffusion method. Sterile cotton swab was dipped in to the prepared inoculum and seeded all over the nutrient agar plate by rotating through an angle of 60°. After each swabbing, the swab was passed round the edges of the agar surface and left to dry for few minutes at room temperature with lid closed. Then with the help of the sterile cork borer (6mm), four wells were made in the labeled inoculated plates. Different concentrations of 100, 75, 50, and 25 mg/ml were dispensed in the respective wells using sterile pipettes. The plates were left for 30mins with the lid closed. The plates were incubated at 37°C for 24hrs then observed for the zones of inhibition which is suggested by the clear area around the well. The experiments were repeated in triplicates and the zones of inhibition were expressed as the mean diameter of the clear zones in millimeters.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentrations (MICs) of the extracts were determined by broth dilution method as described by Malwal and Sarin (2011). One (1) mL of the standardized suspension of the test organism was added to the various concentrations of the extracts in test tubes. The tubes were incubated for 18-24hr at 37°C.The lowest concentration of the spice showing no visible growth was considered as the MIC.

Synergism

In this study, the spices that belong to the same family were combined together in the same ratio (1:1) to determine their combined antimicrobial effect.

Antimicrobial susceptibility testing

The cultures were enriched in nutrient broth for 6 hrs at 37°C. With the help of a sterile cotton swab, the cultures were aseptically swabbed on Muller Hilton agar and allowed to dry. A sterile forcep was used to pick the antibiotic disc (Getamicin-10μg) on the seeded surface of the Muller Hilton agar. The plates were inverted and incubated for 24hrs at

37°C.The diameter of the zones of inhibition was measured to the nearest whole millimeter. The results were interpreted using Clinical Laboratory Standard Institute CLSI (2013) guideline.

Statistical analysis

All the values of zones of inhibition were analyzed using Analyses of Variance (ANOVA). Differences between groups were considered significant at $p<0.05$ levels.

Results

The zones of inhibition of aqueous extract of spices against *Escherichia coli* are presented in figure 1. Ginger, lime leaves, bay leaf, thyme and turmeric showed no antimicrobial effect on *E. coli* while cinnamon had the highest inhibitory effect on *E. coli.* Figure 2 showed the zones of inhibition of aqueous extract of spices against *S. aureus* Onions, turmeric and cinnamon inhibited the growth of *S. aureus.* Red pepper at 100 mg/ml also inhibited the growth of the organism while others did not have any antimicrobial effect on it. The zones of inhibition of aqueous extract of spices against *K. pneumoniae* are presented in figure 3. Turmeric inhibited *K. pneumoniae* at all concentrations followed by cinnamon while red pepper inhibited the organism at 100 mg/mL only. The mean difference between the spices used were highly significant on the test organisms at $p<0.05$.

The zones of inhibition of different ethanol extract of spices against *E. coli* are presented in figure 4. Cinnamon showed the highest inhibitory effect on *E. coli* while curry, bay leaf and ginger had no antimicrobial effect. The effect of the different ethanol extract of spices against *S. aureus* is presented in figure 5. Cinnamon had the highest inhibitory effect followed by red pepper. Lime leaves showed a strong inhibitory effect on *K. pneumoniae* while curry, bay leaf and thyme had no antimicrobial effect on the organism (Figure 6). The mean difference between the spices used were significant on *E. coli* and *S. aureus* but insignificant on *K. pneumoniae* at $p<0.05$.

The synergistic effect of water extract of spices belonging to the same family at different concentration is represented in table 1. Lime - curry had a higher antimicrobial effect against *S. aureus* and *E. coli* while ginger – turmeric did not have any effect on them. The mean difference is significant at $p<0.05$ significant level on *E. coli* and *S. aureus* but insignificant on *K. pneumoniae.*

The synergistic effect of ethanol extract of spices belonging to the same family at different concentration is represented in table 2. Ginger – turmeric have a high inhibitory effect against *S. aureus* and *K. pneumoniae* while Lime–curry has no synergistic effect on *E. coli.* The mean difference is significant at $p<0.05$ on *E. coli* but insignificant on *S. aureus* and *K. pneumoniae.*

Table 3 showed the Minimum Inhibitory Concentration (MIC) of both aqueous and ethanol extracts. Both water and ethanol extract of cinnamon and turmeric displayed the highest minimum inhibitory concentration against the isolates while table 4 showed the antibiotic susceptibility test performed on the isolates using getamicin *E. coli* was highly susceptible to the antibiotic.

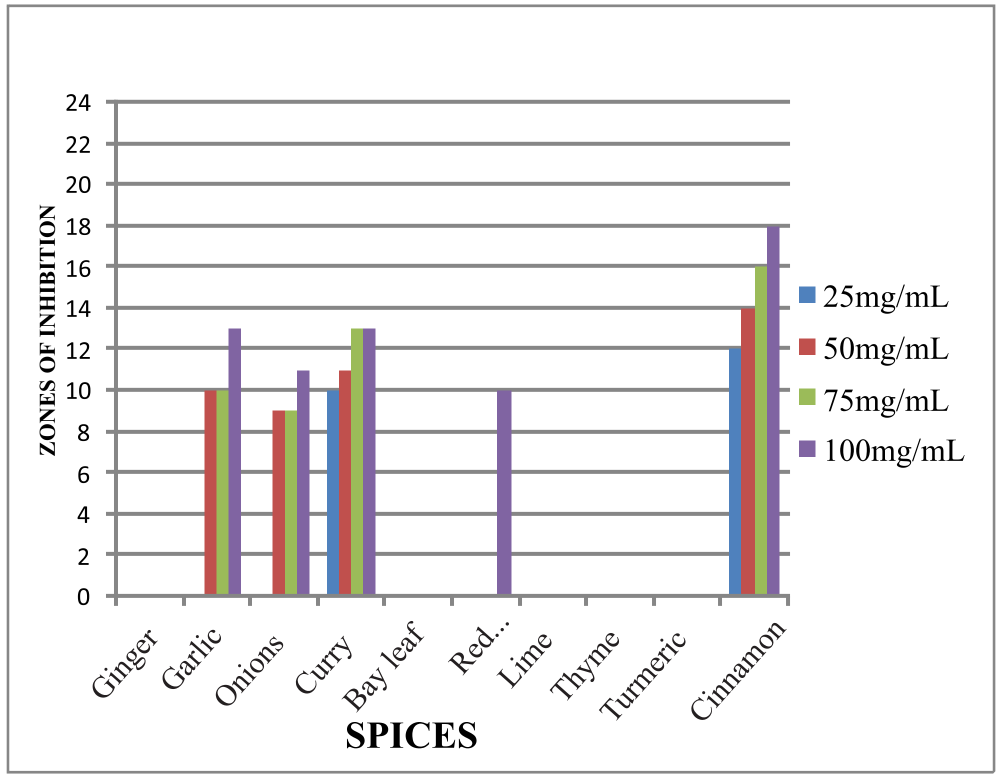

Fig 1: Aqueous extract of spices at different concentrations against Escherichia coli

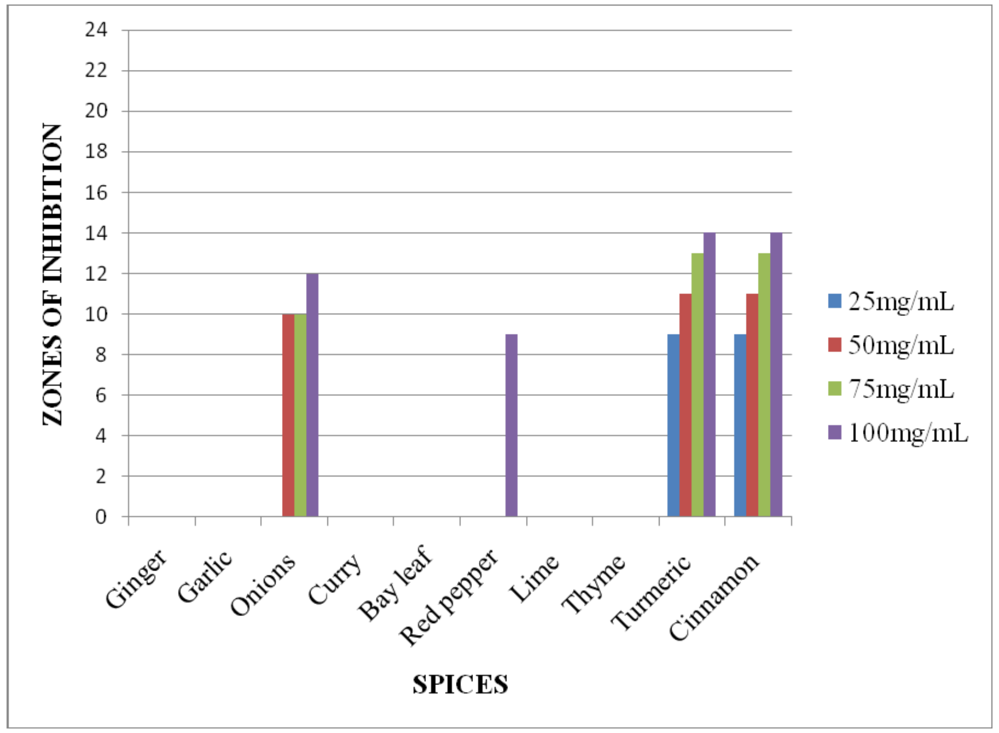

Fig 2: Aqueous extract of spices at different concentrations against *Staphylococcus aureus*

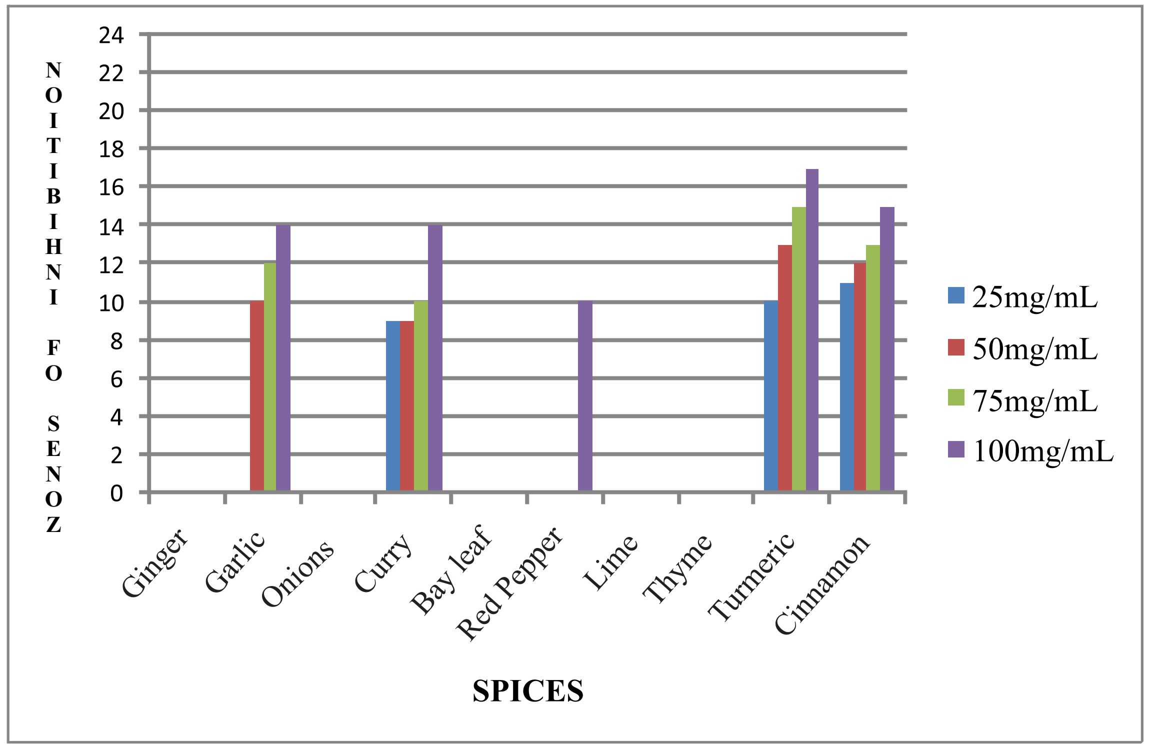

Fig 3: Aqueous extract of spices at different concentrations against Klebsiella pneumoniae

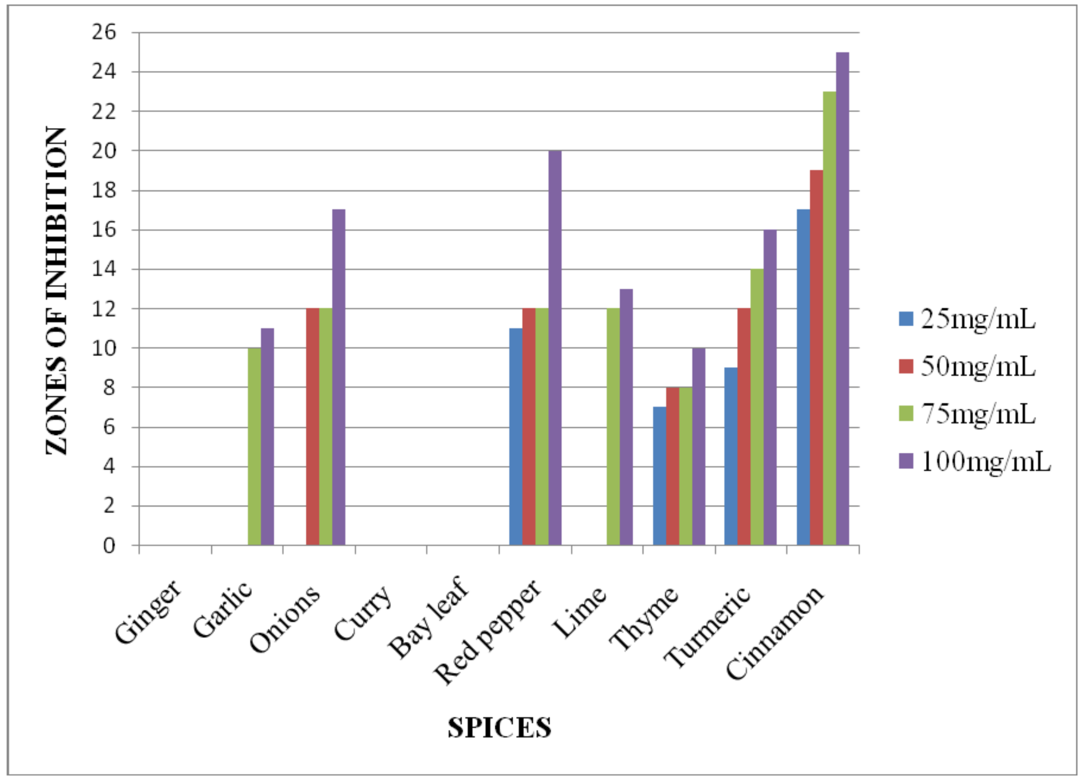

Fig 4: Ethanol extract of spices at different concentration against Escherichia coli

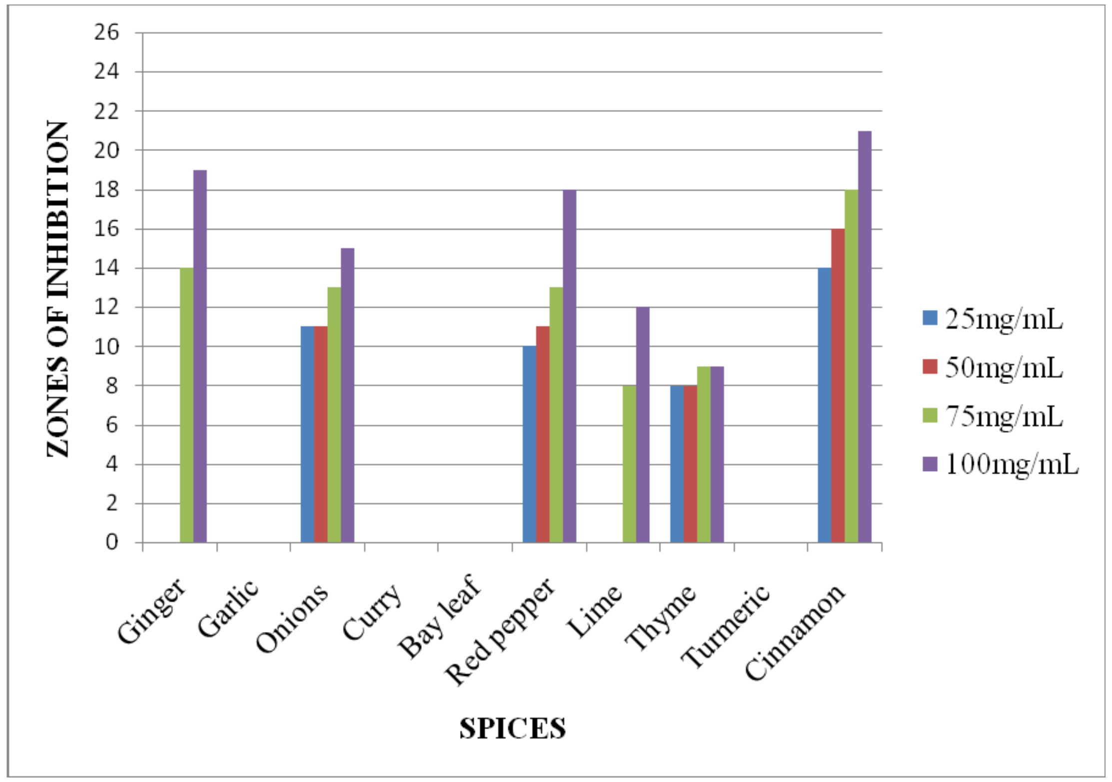

Fig 5: Ethanol extract of spices at different concentration against Staphylococcus aureus

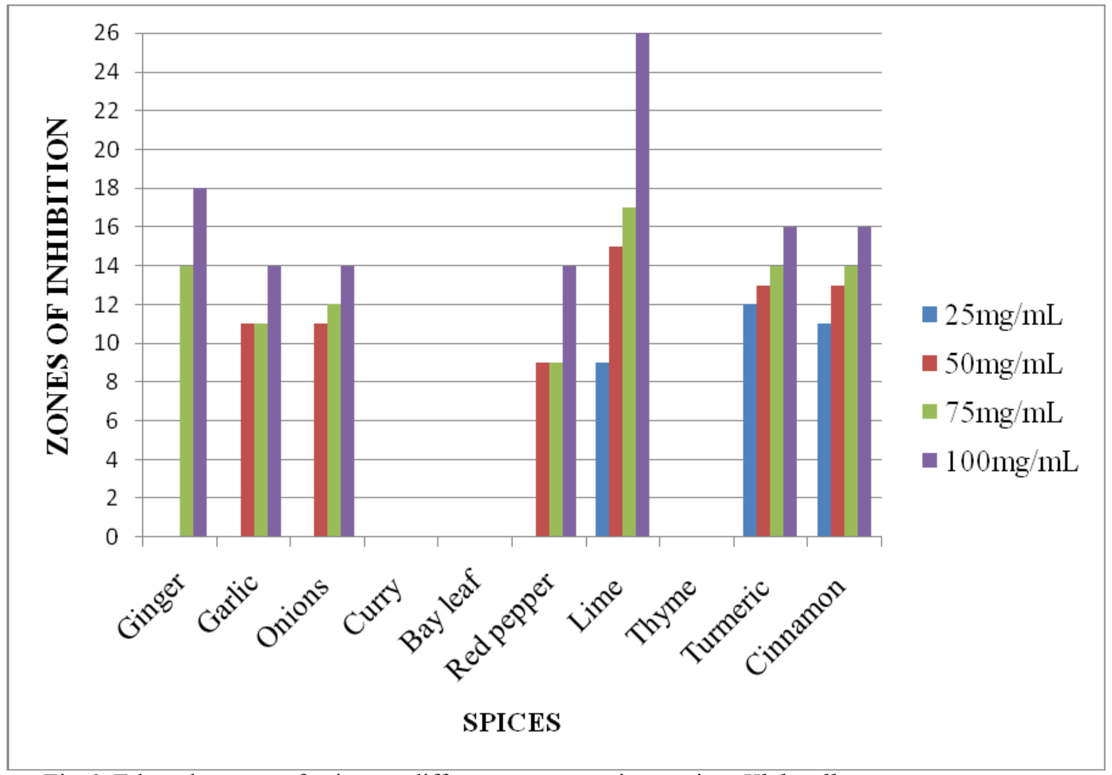

Fig 6: Ethanol extract of spices at different concentration against *Klebsiella pnemoniae*

Table 1: Synergistic effect of aqueous extract of spices of the same family at different concentration against the isolates

Spices extract	*E. coli*				*S. aureus*				*K. pneumoniae*			
(mg/mL)	25	50	75	100	25	50	75	100	25	50	75	100
Garlic - Onion	10	15	16	24	9	10	12	18	10	12	14	18
Lime - Curry	9	10	12	14	-	-	9	12	8	9	12	14
Ginger – Turmeric	-	-	-	-	-	-	-	-	11	13	14	17

Values are means of three replicates.

Table 2: Synergistic effect of ethanol extract of spices of the same family at different concentrations against the isolates

Spice extract	*E. coli*				*S. aureus*				*K. pneumoniae*			
(mg/mL)	25	50	75	100	25	50	75	100	25	50	75	100
Garlic - Onion	9	12	13	14	-	8	9	12	-	-	12	12
Lime - Curry	-	-	-	-	-	-	8	10	9	10	12	15
Ginger - Turmeric	-	8	9	9	8	9	10	12	-	-	9	10

Values are means of three replicates.

***Table 3**: The Minimum inhibitory concentration of aqueous and ethanol extracts*

Spices	Aqueous extract (mg/mL)			Ethanol extract (mg/mL)		
	K. pneumoniae	*S. aureus*	*E. coli*	*K.pneumoniae*	*S. aureus*	*E.coli*
Ginger	-	-	-	-	-	-
Garlic	-	-	-	-	-	-
Onions	-	-	-	-	25	-
Curry	25	-	25	-	-	-
Bay leaf	-	-	-	-	-	-
Red pepper	-	-	-	-	12.5	6.25
Lime leaves	-	-	-	12.5	-	-
Thyme	-	-	-	-	-	-
Turmeric	-	12.5	12.5	12.5	-	6.25
Cinnamon	6.25	12.5	12.5	12.5	6.25	3.125

Table 4: Antibiotic susceptibility test performed on the isolates

Test organism	Zones of inhibition (in mm)
E. coli	25(S)
S. aureus	11(R)
K. pneumoniae	16 (S)

KEY: R – Resistant; S - Susceptible

Discussion and Conclusion

Cinnamon was the most effective spice against the test organisms. The aqueous extract inhibited all the isolates with zones of inhibition between 9-18mm but not as effective as the ethanol extract. The ethanol extract had the highest inhibitory effect against the three isolates in which *E. coli* was the most susceptible with zones of inhibition between 17mm-25mm. This result compared well with the work of Ağaoğlu *et al.* (2007) and Mandal *et al*. (2011).

Aqueous extract of turmeric inhibited *S. aureus* while the ethanol extract did not have any effect. Both aqueous and ethanol extracts have good inhibitory effect on *K. pneumoniae* with zones of inhibition between 10mm-17mm and 12mm-16mm and *E. coli* was resistant to aqueous extract of turmeric but susceptible to the ethanol extract. This is supported by the findings of Chandarana *et al.* (2005) who reported that the extract of turmeric had effect on *E. coli* and suggested that the activity of turmeric is higher when the active compound curcumin is extracted by organic solvents.

All the test organisms were resistant to the aqueous extract of ginger. This may be due to the ineffectiveness of water in extracting the active ingredient in ginger. This is supported by Akintobi *et al.* (2013) who reported that water extract of ginger is ineffective against *E. coli* and has slight inhibitory effect on *S. aureus* while the ethanol extract inhibited *S. aureus* and *K. pneumoniae* with zones of inhibition between14mm-19mm. Onyeagba *et al*. (2005) reported that ethanol extract of ginger inhibited *S. aureus* and *Bacillus* sp. *E. coli* was completely resistance to all extracts of ginger. This contradicts the work of Indu *et al.* (2006), Yusha'u *et al*. (2008) and Witkowska *et al*. (2013) who reported that ethanol extracts of ginger showed remarkable activity against *E. coli.*

Aqueous extract of curry inhibited *E. coli* and *K. pneumoniae* with zones of inhibition between 10-13mm and 9-14mm respectively while S. aureus was not sensitive. Ethanol extract of curry showed no activity against the isolates and this is in contrast with the findings of Das *et al.* (2012) who reported that *E. coli* and *S. aureus* were sensitive to ethanol extract of curry leaf. This result contradicts the study of Malwal and Sarin (2011) who reported that *S. aureus* and *E. coli* were not inhibited by aqueous extract of the root of *Murraya koenigii* L.

The aqueous extract of lime showed no activity against the test organisms. However, ethanol extract of lime leaves showed inhibitory effect against the bacteria especially against *Klebsiella pneumoniae* with zone of inhibition between 9-26mm while it has lesser effect on *E. coli* and *S. aureus*. Khan Pathan *et al.* (2012) reported that lime leaf showed a higher activity on *Klebsiella pneumoniae* and *S. aureus* but that *E. coli* was not affected by the extract.

The aqueous extract of red pepper only inhibited the isolates at 100 mg/mL while the ethanol extract had excellent effect on all the isolates at all concentrations. This is in line with the work of Soetarno *et al.* (2009) but in contrast with the work of Ağaoğlu *et al.*(2007) who reported that red pepper had no antimicrobial effect on the test organisms.

Both the aqueous and ethanol extract of bay leaf showed no activity against the three bacterial isolates. This result is in contrast with the findings of Chaudhry and Tariq (2006) who reported that *E. coli* and *K. pneumoniae* were sensitive to aqueous extract of bay leaf. Also, Witkowska *et al.* (2013) reported that ethanol and water extract of bayleaf showed activity against *E. coli* and *S. aureus.*

E.coli and *K. pneumoniae* were inhibited by both ethanol and aqueous extracts of garlic while *S. aureus* was not sensitive to both extracts at all concentrations. This is in line with the findings of Ojo *et al.* (2007) and Joe *et al.* (2009) who reported inhibition of *E. coli, S. aureus* and *K. pneumoniae* by ethanol extract of garlic. Eja *et al*.(2011) also reported that *E. coli* and *S. aureus* were inhibited by garlic. There was no difference between the aqueous and ethanol extract. This result is similar to the findings of Safithri *et al.* (2012) who reported that the antibacterial activity of ethanol extract of garlic was mild compared to the aqueous extract and that *S. aureus* was not inhibited by ethanol extract.

Aqueous extract of onion displayed no activity against *K. pneumoniae.* Ethanol extract of onion showed antimicrobial activity against E. coli, *S. aureus* and *K. pneumoniae* with zones of inhibition ranging from 11mm-17mm.This is in agreement with Witkowska *et al.* (2013)who reported that *E. coli* and *S. aureus* were sensitive to the ethanol and aqueous extracts of onion.

All the three test organisms were not sensitive to the aqueous extract of thyme while the ethanol extracts showed weak inhibitory effect on *E. coli* and *S. aureus* but *K.pneumoniae* was not sensitive to the ethanol extract. This is supported by the work of Witkowska *et al.* (2013) who reported that ethanol extract of thyme displayed weak inhibitory properties against *S. aureus* and *E. coli.* Sağdıç (2003) in his work reported that thyme hydrosols showed high inhibitory effect against *E. coli* and *S.*

aureus. Garlic and onion aqueous extract showed compensatory synergism with excellent inhibition zone of 10mm-24 mm against the three bacterial isolates which is higher than the inhibition zones reported for the individual garlic and onion aqueous extracts while the ethanol extracts showed antagonistic synergism with inhibition zones ranging from 9 mm-14 mm against the three clinical bacterial isolates. These are lower compare to 11 mm-17 mm and 11mm -14 mm reported for ethanol extract of onion and garlic respectively.

Lime and curry aqueous extract showed an inhibition zone of 9 mm-14 mm against the three bacterial isolates. Aqueous extract of lime has no effect on the isolates. There is no synergism observed in the spices while the ethanol extracts displayed antagonistic synergism with an inhibition zone of 8 mm-15 mm for *S. aureus* and *K. pneumoniae* with no activity on *E. coli* which is lower compare to ethanol extract of lime which have an inhibition zone of 8 mm-19 mm on the three bacterial isolates although curry ethanol extract have no activity on the three clinical bacterial isolates.

Ginger and turmeric aqueous extract showed an antagonistic effect with inhibition zone of 11 mm-17 mm against *K. pneumoniae* and no activity against *E. coli* and *S. aureus* which is lower than 9 mm – 18 mm reported for turmeric only while the ethanol extract also showed antagonistic effect with inhibition zone of 8 mm-12 mm against the three clinical bacterial isolates which is lower when compared to 14 mm-19 mm and 9 mm-16 mm reported for ginger and turmeric respectively.

The result of the MIC revealed that ethanol and aqueous extract of cinnamon showed remarkable effect on all the isolates in the range of 3.125 – 12.5 mg/mL and 6.25 – 12.5 mg/mL respectively. The ethanol extract was more effective than the aqueous extract.

For antibiotic sensitivity test, *E. coli* was more sensitive to getamicin followed by *S.aureus* while *K. pneumoniae* was resistant. Khan Pathan *et al*. (2012) reported zones of inhibition for *E. coli, K. pneumoniae* and *S. aureus* as 10 mm, 15 mm and 14 mm respectively for Getamicin. Akintobi *et al.* (2013) reported that *S. aureus* and *E. coli* were resistant to getamicin.

This research has showed that spices extracts possess different antibacterial activities which can be exploited in the treatment of diseases instead of their use only as flavor or nutrient supplement. This is particularly of urgent interest when the growth rate of multi-resistant drug strains of bacteria worldwide is considered (Prescott et al., 2008).

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