

# Role of African spices against *Escherichia coli* isolated from potable water sample in Sokoto, Nigeria

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## ABSTRACT

The antibacterial activity of five selected spices against *Escherichia coli* was carried out using agar well diffusion method. Two solvent were used for extraction (ethanol and hot water), the extract were prepared at different concentration (20, 30, 40, 50 and 90 mg/ml). At 90 mg/ml concentration of ethanol extracts *Zingiber officinale* (11 mm), *Allium sativum* (9.0 mm), *Syzygium aromaticum* (5 mm) and that of hot water extracts the zone of inhibition were; *Z. officinale* (5.0 mm), *A. sativum* (4.0 mm), *S. aromaticum* (2.0 mm) while *Xylopi aethiopica* and *Piper umbellatum* do not showed any activity against the *E. coli* of both ethanol and hot water extracts. The standard of seven antibiotics used (control) for comparison only ciprofloxacin was observed to be effective against *E. coli*. The autoclave extracts do not have any activity against the test organism even at high concentration. The minimal inhibitory concentration and minimal bactericidal concentration were also determined. We observed that the ethanol extracts were more affective against the *E. coli* than that of hot water extracts. The phytochemical analysis showed the presence of tannins, flavonoids, saponins, steroids and alkaloids in varying concentration.

**Keywords:** Africa, spices, *Escherichia coli*, isolated water.

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## INTRODUCTION

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential and contain anti-microbial substances (Doughari et al., 2008). The used of plants have made a great contribution to human health and thus, a majority of the world's population in developing countries still relies on herbal medicine to meet their need (WHO, 1991). The World Health Organization has estimated that up to 80% of the World's population rely on plants for their primary health care, in Nigeria WHO survey in 1983 estimated that up to 75% of the population patronizes traditional medicine (Okwute, 1992). Spices have been traditionally used as coloring agents, preservative and flavor which give food appetite for human consumption, therefore the safety of food products and shelf life depends on the quality and quantity of spices added to the food products (Okigbo and Omodamiro, 2006).

The spices used in this study are: *Allium sativum* (Garlic), *Zingiber officinale* (Ginger), *Xylopi aethiopica*,

*Syzygium aromaticum* and *Piper umbellatum*. The medicinal value of these spice are believed to be due to the presence of some chemical substances that have define physiological action in and on the human body. The most important of these substances are alkaloid, tannins, steroids, flavanoids, saponins, etc (Cowan, 1999).

*A. sativum* commonly known as garlic belongs to the species of onion family Aliaceae. Garlic (*A. sativum*) is one of those plants that were seriously investigated over the years. It has been used for centuries to fight infections (Onyeagba et al., 2004). In 1958, Louis Pasteur first observed garlic antibacterial activity and it was used as an antiseptic to prevent gangrene during World War 1 and World War 2 (Groppa et al., 2007). Since then, research had demonstrated its effectiveness against bacteria, protozoa, fungi and some viruses (Jaber and Al-Mossawi, 2007). Previously conducted researches confirmed that garlic is not only effective against many

Gram positive and Gram negative bacteria but also possess antiviral and antifungal activity (Martin and Ernst, 2003). *Z. officinale* commonly known as ginger, belong to the family Zingiberaceae (Sharma et al., 2010). Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections (Tan and Vanitha, 2004). Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It has also "Generally Recognized as Safe" (GRAS) by the US FDA. *X. aethiopica* commonly known as Ethiopia or Negro pepper and belong to the family of Annonaceae. Nadia et al. (2013) reported that *X. aethiopica* possesses nutritional and medicinal value. The powdered bark of the tree is dusted onto ulcerous wounds, while a decoction of the leaves and roots is a general tonic for fever in Nigeria (Burkil, 1999). *Syzygium aromaticum* is also called *Eugenia aromaticum* or *Eugenia caryophallata* commonly called clove. Clove is aromatic dried flower bud of a tree in the family Myrtaceae (Chaieb et al., 2008). Clove are used in Chinese and Western herbalist, clove are as carminative to increase hydrochloric acid in the stomach and to increase paristalsis (Phyllis and James, 2000). The oil extracted from clove is used for acne, wart, scars and parasite. Research has shown that clove oil is used as topical application to relieve pain and promote healing (Chaieb et al., 2007). *P. umbellatum* belong to the family of Piperaceae. *P. umbellatum* are well known to the indigenous communities of Sabah, they are widely used as herbal medicines and food (Das et al., 1996).

Interest in this area has increased tremendously due to some of the limitations associated with the used of synthetic antimicrobial agents. Role of spices against *E. coli* are not well establish in Africa hence, this study aim to investigate the effect of African spices against *E. coli* isolated from potable water sample.

## METHODOLOGY

Five dried spices: *A. sativum*, *Z. officinale*, *X. aethiopica*, *P. umbellatum* and *S. aromaticum* were obtained from Sokoto central market. The spices were washed, grounded and sieved to obtained fine powder. Water sample were collected from three different places (In campus metropolis, Kwalkwalawa and in town) and transported to the Microbiology Department, Usman Danfodiyo University Sokoto, Nigeria.

### Sample identification

The five spices were identified and authenticated at herbarium unit, department of Biological Science Usman Danfodiyo University Sokoto, Nigeria. The voucher numbers are as follows: *A. sativum* Vn (014), *Z. officinale* Vn (010), *X. aethiopica* Vn (013), *P. umbellatum* Vn (012), *S. aromaticum* Vn (010).

### Media preparation

All media to be used were prepared according to manufacturers' instruction contained on the label containers. The media are Eosin

methylene blue (EMB) and nutrient agar and other biochemical media such as peptone water, Kovac's reagent, Glucose phosphate broth, Methyl red reagent, Simmon's media and biochemical tests like indole test, methyl red test, Voges-Proskauer test, Urease test, Motility test, Sugar fermentation test, Oxidase test are carried out for identification of test organism.

### Inoculation

Inoculation was done by using spread plate technique, after processing the water sample (Serial dilution) 0.1 ml of water was taken from the fourth and fifth test tube and poured it into EMB medium and sterile bent glass rod was used to spread the inoculums throughout the surface of the medium. The plate was then incubated at 37°C for 24 h. The resulting isolates were transferred into fresh prepared nutrient agar slants to be preserved for further identification.

### Extraction using ethanol and water

The extraction was carried out using the methods of Okigbo et al. (2005) with some modification. Five spices were washed with sterile distilled water and dried at room temperature. Then, grounded to fine powder using pestle and mortar and sieved. The powders were stored at room temperature in a dry container.

Two solvent were used for extraction, that is, ethanol extraction and hot water extraction (aqueous extraction). This was done by weighing 25 g of fine powder of spices and soaked into 200 ml of both ethanol and hot water, and allowed to stand for 24 h. It was then filtered using Whatman no. 1 filter paper. The resulting filtrates were concentrated by evaporation and dried in a drying cabinet at 40°C.

### Preparation of extract in varying concentration

The two extracts obtained (both ethanol and hot water extracts) were weighed out using weighing balance into 0.1, 0.15, 0.2, 0.25 and 0.45 g, respectively. The extracts were dissolved into 5 ml of sterile distilled water contained in a set of test tube. The preparation was vortexes thoroughly to dissolve and allowed to stand for about 2 h. The extracts concentrations were 20, 30, 40, 50 and 90 mg/ml, respectively.

### Sensitivity testing

Antibacterial activity test was done using agar in accordance of Perez et al. (1999) with some modifications. This was done by making a bore on the nutrients agar medium, at a distance of 10 mm. 2 ml from the prepared extracts concentrations were mixed with 1 ml of prepared plane agar contained in the sets of test tube and mixed gently. About 0.5 ml of the preparations were aseptically poured into the well made on the nutrient agar medium, the plate were then incubated at 37°C for 24 h, and observed for the clearing zone or zone of inhibition. The standards of seven antibiotics were used to determine the susceptibility testing against the *E. coli* as a comparison of spices antibacterial activity. The antibiotics that are used were: Clindamycin (CC), Ciprofloxacin (CPX), Gentamycin, Tetracycline, Amoxicillin (AM), Streptomycin (S) and Cloxacillin (CX).

### Autoclaved extracts

The autoclaving of the spices extracts were carried according to method used by (Saravasta et al., 2010). The concentrations of the

**Table 1.** The amount of the extracts yield for both ethanol and hot water extracts.

Spices	Amount used for extraction (g)	Yield of extracts (g)	
		Ethanol	Hot water
<i>Allium sativum</i>	100	13.34	9.53
<i>Xylopi aethiopica</i>	100	9.23	7.63
<i>Syzygium aromaticum</i>	100	10.45	9.22
<i>Zingiber officinale</i>	100	12.34	10.80
<i>Piper umbellatum</i>	100	8.21	8.90

extract (20, 30, 40, 50 and 90 mg/ml) of ethanol extracts were autoclaved for 15 min at 121°C. About 0.5 ml of each were poured aseptically into the well made on the nutrient agar, the plate were then incubated for 24 h at 37°C and observed for the clearing zone

#### Determination of minimal inhibitory concentration (MIC)

Determination of minimal bactericidal concentration (MBC) of crude extracts against the test organism was determined in accordance of Spencer and Spencer (2004) with modifications. This was done by preparing concentrations employing nutrient broth as diluents, the concentration are 90, 70, 50, 40, 30, 20 and 10 mg/ml, respectively. The concentration were aseptically inoculated with the test organism and incubated at 37°C for 24 h. After which the broth were observed for the presence or absence of growth. The minimal inhibitory concentration was taken as the lowest concentrations that prevent the growth of the test organism. While in MBC, sample were taken from the broth with no visible growth in the MIC assay and subculture on freshly prepared nutrient agar and incubated at 37°C for 24 h. The MBC was taken as the concentration of the extracts that did not show any visible growth on a new set of agar plates.

#### Phytochemical screening

The phytochemical tests were carried out in accordance of Sofowora (1999). Both ethanol extracts and the hot water extracts was screen quantitatively for the presence of tannins, steroids, alkaloids, saponins and flavonoids.

## RESULTS

In this research conducted to access the antibacterial activity of five selected natural spices against *E. coli*, the following results were obtained and are presented in Tables 1 to 7.

It is observe that ethanol yields more extract then the hot water as seen in Table 1. The standard of seven antibiotics was used and result presented in Table 2. It was observed ciprofloxacin was very effective against *E. coli*.

It was observed that activity of ethanol extracts was more active against the *E. coli*. At concentration of 90 mg/ml base on inhibition zone of *Z. officinale* (11.0 mm) and *A. sativum* (9.0 mm) followed *S. aromaticum* (5.0 mm), while *P. umbellatum* and *X. aethiopica* did not showed any activity against *E. coli*. Also, no activity at low concentration except *Z. officinale* showed 2.0 mm at

**Table 2.** The antibiotic sensitivity testing as control.

S/N	Antibiotics	Zone of inhibition (mm)
1.	Ciprofloxacin(CPX)	21
2.	Gentamycin (G)	0
3.	Clindamycin (CC)	0
4.	Amoxicillin (AM)	9
5.	Streptomycin (S)	15
6.	Cloxacillin (CX)	0
7.	Tetracyclin (T)	0

concentration 30 mg/ml (Table 4).

It was observed that hot water extracts was active at concentrations of 50 and 90 mg/ml while inactive at lower concentrations. At 90 mg/ml the *Z. officinale* shows 5.0 mm, *A. sativum* (4.0 mm) and *Syzygium aromaticum* (2.0 mm) while no activity was observed on *X. aethiopica* and *P. umbellatum* even at high concentration (Table 5). We observed that the extracts showed no activity both in low, medium and at high concentration.

Minimal inhibitory concentration and minimal bactericidal action of each of the spices at different concentration showing clear inhibition was determined on ethanol extracts and result is presented in Table 6.

The result of phytochemical tests carried out on both ethanol and hot water extracts of the five spices was presented above. They include tannins, saponins, steroids, alkaloids, flavonoids.

## DISCUSSION

The assessment of antibacterial activity of five selected natural spices against *E. coli* was carried out using two solvent (ethanol and hot water) extractions and agar well diffusion method. From the result obtained they were found out to be effective inhibitors of the *E. coli* especially *Z. officinale* and *A. sativum*.

The extract yields of ethanol were higher than that of aqueous and also the activity of ethanol extract was higher than that of aqueous extract. This may be because ethanol is an organic solvent and will dissolve many organic substances adequately, hence liberate the active compound required for antimicrobial activity.

**Table 3.** Results of antibacterial activity of ethanol extracts of five selected natural spices.

Spices	Zone of inhibition (mm)/Concentration				
	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	90 mg/ml
<i>Allium sativum</i>	0	0	4	4	9
<i>Xylopi aethiopica</i>	0	0	0	0	0
<i>Syzygium aromaticum</i>	0	0	2	3	5
<i>Zingiber officinale</i>	0	4	4	6	11
<i>Piper umbellatum</i>	0	0	0	0	0

**Table 4.** Present the antibacterial activity of hot water extract against *E. coli*.

Spices	Zone of inhibition (mm)/Concentration				
	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	90 mg/ml
<i>Allium sativum</i>	0	0	0	2	4
<i>Xylopi aethiopica</i>	0	0	0	0	0
<i>Syzygium aromaticum</i>	0	0	0	0	2
<i>Zingiber officinale</i>	0	0	3	3	5
<i>Piper umbellatum</i>	0	0	0	0	0

**Table 5.** Antibacterial activity of autoclaved extracts of both methanol and hot water extracts against *E. coli*.

Spices	Zone of inhibition (mm)/Concentration				
	20 mg/ml	30 mg/ml	40 mg/ml	50 mg/ml	90 mg/ml
<i>Allium sativum</i>	0	0	0	0	0
<i>Xylopi aethiopica</i>	0	0	0	0	0
<i>Syzygium aromaticum</i>	0	0	0	0	0
<i>Zingiber officinale</i>	0	0	0	0	0
<i>Piper umbellatum</i>	0	0	0	0	0

**Table 6.** Results showing the inhibitory action of each spice at different concentrations.

Spices	Dilution rate/Concentration (mg/ml)						
	90 mg/ml	70 mg/ml	50 mg/ml	40 mg/ml	30 mg/ml	20 mg/ml	10 mg/ml
<i>Allium sativum</i>	-/-	-/-	-/-	-/-	+	+	+
<i>Xylopi aethiopica</i>	Nc						
<i>Syzygium aromaticum</i>	-/-	+	+	+	+	+	+
<i>Zingiber officinale</i>	-/-	-/-	-/-	-/-	-/+	+	+
<i>Piper umbellatum</i>	Nc						

(-) = No visible growth, (+) = Visible growth, (Nc) = Not conducted.

**Table 7.** Results of phytochemical analysis of the compounds.

Spices	Phytochemical components of ethanol/hot water extracts				
	Flavonoids	Tannins	Steroids	Saponins	Alkaloids
<i>Allium sativum</i>	-/-	-/-	+++	++	+++
<i>Xylopi aethiopica</i>	-/+	-/-	+/+	+/+	+/+
<i>Syzygium aromaticum</i>	++/+	++/-	+/+	++/-	+/+
<i>Zingiber officinale</i>	-/-	-/-	+++	+++	++
<i>Piper umbellatum</i>	-/-	-/-	+/+	+/+	++

(-) = negative, (+) = positive, (+++/++) = strongly positive.

The standard of seven antibiotics were used as comparison to spices antibacterial activity. It was found that *E. coli* were resistant to four antibiotics (gentamycin, clindamycin, cloxacillin and tetracycline). This may be due to the *E. coli* nature, cell structure; gram negative has outer membrane which blocks the penetration of antibiotics including the extracts of spices making them resistant (Indu et al., 2006). The antibacterial activities of various spice extracts found in this work were dependent on concentration of the extracts against the *E. coli*. For instance, at higher concentration 90 mg/ml of ethanolic *Z. officinale* extracts inhibited *E. coli* with 11.0 mm and the same concentration *A. sativum* inhibited *E. coli* with 9.0 mm and *S. aromaticum* showed 5.0 mm at the same concentration, while *X. aethiopica* and *P. umbellatum* did not show any activity even at higher concentration. Similarly, none of the spices show any activity at lower concentration.

The antibacterial activity were not observed from all the spices at lower concentration, rather at 90 mg/ml concentration the *Z. officinale* extracts showed 5.0 mm zone of inhibition, *A. sativum* 4.0 mm and *S. aromaticum* 2.0 mm while *X. aethiopica* and *P. umbellatum* did not showed activity against *E. coli* even at higher concentration. The mechanism responsible for the inhibition of the bacterium includes enzyme inhibition by oxidized compound, possibly through reaction with sulfhydryl group (Manson and Wasserman, 1987). Similarly allicin as the active ingredient in garlic act by partially inhibiting DNA and proteins synthesis and also totally inhibiting RNA synthesis as their primary target. The gingerol-related components of ginger do exert their action by inhibiting protein synthesis and thus, have been reported to possess antibacterial and antifungal properties (Onyeagba et al., 2004). The insensitivity or relatively very low activity of some spices extracts against the test organism implies that the *E. coli* possess a reservoir of resistant gene and efficiently exchange genetic material with other pathogens such as *Salmonella*, *Kleibsell*a and *Vibrio* species as such confer resistance from one agent to another (Leavy et al., 2008). The result from recent study revealed that the ethanol extracts are effective inhibitor of microorganism. This implies that the ethanol extract was much better and powerful, mainly due to better solubility of active compound in organic solvents (De Boer et al., 2005).

Spices are naturally used to treat various ailments resulting from microbial and non-microbial origin. The results of this study suggest that these spices contribute significantly to inhibit microorganism which gives credence to use these spices in the treatment of the diseases cause by bacterium. The non-activity of *X. aethiopica* and *P. umbellatum* against *E. coli* confirms previous report (Okibgo et al., 2005) and further suggests that *X. aethiopica* will not be useful in the treatment of diseases caused by *E. coli* (Karioti et al., 2004).

The antibacterial activity of the hot water extract

showed very low activity against *E. coli*. This is because a component from the spices active against *E. coli* was not properly extracted by the hot water in contrast to ethanol extracts. Nearly all identified compound from the plant active against microorganism are aromatic or saturated compound they are often obtained through methanol or ethanol extraction with the exception of water soluble compound such as polypeptides and polysaccharide are commonly affective to inhibit the pathogens e.g. virus (Martin, 2004). Hence, important active components from the spices may not be adequately extracted by hot water extraction procedure.

This implies that water (hot water) extraction is another possible alternative employing as solvent and thus, is useful to inhibit other microorganism. The insensitivity or very low activity of hot water extract against the *E. coli* investigated in this study is in agreement with previous work, which showed that aqueous extracts of plant generally showed little or no activity (Koduru et al., 2006).

The autoclaved extracts of both ethanol and hot water extracts do not showed any antibacterial activity against the *E. coli* even at high concentration. This might be probably due to deterioration and denature of certain proteins and other active constituent of the spices as a result of high temperature and pressure in the autoclave. This implies that the spices possess secondary metabolite and other constituents capable of inhibiting the growth of microorganism, these constituents are unstable at high temperature and pressure and thus might undergoes deterioration as a result of high temperature in the autoclave. The non-activity of the autoclaved extracts is in agreement with previous work which showed that autoclaved extracts of both ethanol and water extracts do not have any antibacterial activity (Saravanan et al., 2010).

Phytochemical screening of the five spice extracts (*A. sativum*, *Z. officinale*, *X. aethiopica*, *P. umbellatum* and *S. aromaticum*) showed tannins, flavonoids, saponins, steroids and alkaloids to be present in varying amount. This compounds are known to be biologically active and therefore aid in the antimicrobial activity; these secondary metabolite exert activity through different mechanism. Tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the proteins synthesis, tannins are known to reacts with proteins to provide a typical tanning effects which is important for the treatment of inflamed or ulcerated tissue, tannins also have proteins binding capacity with the bacterial proteins through so called non-specific force such as hydrogen binding and hydrophobic effects as well as by covalent bond formation (Shimada, 2006). Flavonoids play an important role due to their ability to complex with bacterial cells (Tsuchiya et al., 1996). Steroidal compound presence in plants extracts are important and interest due to the relationship with various anabolic hormones, the mechanism of action of steroid are not fully understood but speculated to involved

membrane destruction by lipophilic compounds (Okwu, 2001). One of the most compound biological properties of steroid is their toxicity against the cells of foreign organism. This activity is widely studied for their potential uses in the elimination of human cancer cell line (Nobori et al., 1994). Alkaloid also is the largest group of phytochemical in the plant has amazing effect on human and has led to the development of analgesics (Kam and Liew, 2002). Saponin is found to be present in spices (Just et al., 1998). This implies that the inhibition of the *E. coli* by the extracts could be as a result of one of these active components or in combination. Since ethanol extracts was found active against the *E. coli*, it can be refined as antibiotic to cure diseases caused by *E. coli* such as gastroenteritis and urinary tract infection (UTI). It can also be made as ointment to be applied on the genital of patient infected with UTI.

## CONCLUSION

This research showed that *E. coli* are susceptible to both ethanol and hot water extracts of African spices at different concentration especially at 90 mg/ml. The activity of ethanol extracts of these spices are arranged in decreasing order as follows: *Z. officinale* (11.0 mm) > *A. sativum* (9.0 mm) > *S. aromaticum* (5.0 mm); and that of hot water extracts are: *Z. officinale* (5.0 mm) > *A. sativum* (4.0 mm) > *S. aromaticum* (2.0 mm), while *X. aethiopica* and *P. umbellatum* did not show any activity even at high concentration. The extracts of these spices could be a possible source to obtain new and effective herbal medicine to treat diseases caused by *E. coli* in the community. However, it is necessary to determine their toxicity, side effects and pharmaco-kinetic properties.

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