

Preliminary evaluation of antibacterial potential of four common Nigerian plants against isolates of *Staphylococcus aureus* and *Escherichia coli*

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Abstract: The antibacterial activity of water and ethanol extracts of *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Zingiber officinale* (Ginger), *Allium cepa* (Onions) were evaluated against two bacteria (*Staphylococcus aureus* and *Escherichia coli*). The ethanol and water (cold and hot) extracts of these plants were obtained by standard pharmacognostic methods while the antibacterial activities were determined by modified agar well diffusion method. Ampicillin was used as the control antibiotic. The cold water extract of *A. indica* showed no antibacterial activity while the hot water and ethanolic extracts of *A. indica* showed maximal antibacterial activity with inhibition zone diameters (IZD) of ethanolic extract at 27mm for *S. aureus* and 40mm for *E. coli* while hot water extract at 18mg/ml showed 30mm for *E. coli* and 27mm for *S. aureus*. Fresh ethanolic extracts of *A. sativa* at 0.5mg/ml showed 28mm as maximal IZD against the test bacteria while the fresh extract of *Z. officinale* showed 18mm maximal IZD against *S. aureus* but no IZD against *E. coli*. The fresh ethanolic extract and aqueous (cold water and hot water) of *A. cepa* (onions) produced no antibacterial activity against the tested bacteria at all concentrations. The test bacteria were resistant to ampicillin (control) at all concentrations used in this study. Findings from this study show that two (*A. indica* and *A. sativum*) of the plants constitute significant antibacterial activity against *S. aureus* and *E. coli*. However, plants with significant antibacterial activity should be subjected to further analysis in order to unravel the active ingredients responsible for their antibacterial actions.

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Introduction

Herbal remedies are widely known to be used in the treatment of many infectious microorganisms. Plant materials continue to provide a major source of natural therapeutic remedies and play an important role many health care system of developing countries (Czygan, 1993).

In recent time, there is increase cases of side - effects associated to synthetic antibiotics and suboptimal antimicrobial efficacy (Corazo *et al.*, 1999). Hence, justify the need to search for safer and more effective antibiotics from natural plant products. These was possibly due to the presence of various bioactive ingredients of these plants that could exhibit antibacterial properties. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005) and this calls for the development of drugs made from herbal sources. Traditional healers claim that some medicinal plants (such as *Newbouldia laevis*, *Zingiber officinale*, *Azadirachta indica*, *Allium sativum* amongst others) are more efficient in treating infectious diseases than

synthetic antibiotics (Shah, 2005). It is necessary to evaluate the potential use of these folk medicine for possible consideration as alternative treatment of microbial pathogens.

A. indica (neem) is an herbal plant widely distributed during all seasons. Each part of neem tree has some medicinal property. Neem leave, bark extracts and neem oil are commonly used for therapeutic purpose (Ketkar and Ketkar, 1995). Neem oil suppresses several species of pathogenic bacteria such as *staphylococcus aureus* and *S. typhi*, all strains of *Mycobacterium tuberculosis* (Iqba *et al.*, 2008). A recent study conducted (Jahan *et al.*, 2007) showed 92% susceptibility of *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Escherichia coli*, *Proteus spp* and *Klebsiella spp* to neem oil. In India, the neem tree is still regarded as "village dispensary". More so, another study showed that while extracts of *A. indica* consistently killed *S. aureus* at a concentration of 0.5mg/ml; it had little activity on *E. coli* (Okemo *et al.*, 2001).

Garlic (*A. sativum*) has traditional and medicinal applications as an anti-infective agent (Lawson,

1998). In vitro evidence of the antimicrobial activity of fresh and freeze dried garlic extracts against many bacteria, fungi, and viruses support these applications. Allicin is formed when garlic cloves are crushed. Methyl and alkyl sulphide derivatives of allicin are formed by the steam distillation of mashed garlic to produce garlic oil, which is used in many medicinal garlic products.

Cavallito *et al* (1945) attributed the antibacterial properties of garlic clove homogenates to allicin. These properties were confirmed against *E. coli* and *S. aureus* for garlic cloves homogenates plus related garlic compounds and commercial supplements.

The medical form of ginger historically was called "Jamaica ginger"; it was classified as a stimulant and carminative, and used frequently for dyspepsia and colic. Ginger has also been used to treat some cases of diarrhea, Nausea etc. Zingerone, an active component of ginger is likely to be the active constituent against enterotoxigenic *Escherichia coli* heat-labile enterotoxin induced diarrhea (Chen *et al.*, 2007).

Ginger (*Zingiber officinale*) is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (Ali, 2008).

There are several claims have been made for the effectiveness of onions against conditions ranging from the common cold to heart disease, diabetes, osteoporosis, and other disease. Onions have also been used in the United States for the treatment of topical scars (Zurada *et al.*, 2006).

In view of these, the present study sought to determine the antibacterial activity of plant extracts (*N. laevis*, *C. olitorus*, *A. indica*, *A. sativum*, *Zingiber officinale*, *Allium cepa*) in the form of zone of bacterial growth inhibitions on pure isolates of *E. coli* and *S. aureus*.

Materials And Method

Test micro-organisms

Pure cultures of *Escherichia coli* and *Staphylococcus aureus* were taken from isolated specimens which exhibited resistance to some antibiotics in hospitalized patients at Ebonyi State University Teaching Hospital. They were taken based on ethical clearance approval from the ethical committee in the hospital. The bacteria were cultured over-night (18–24 h) at 37 °C on nutrient broth for the preparation of cell suspensions. The bacteria cell suspensions were homogenized and adjusted to 0.5 McFarland standards (5×10^5 CFU/mL) using spectrophotometry.

Preparation of plant extracts

The plant extracts were prepared using the modified method of Alade and Irobi (Alade *et al.*; 1993). The plants (*A. indica*, *A. sativum*, *A. cepa* and *Z. officinale*) were grinded into powdered form using sterile electric blender, after drying them in the hot air oven at ambient temperature. Thereafter, 60g of individual dried powdered plants were soaked separately in 300ml of cold water, 300ml hot water and 300ml ethanol for 72 hours at 35°C. Each mixture was refluxed and agitated. Then the filtrates were obtained by filtration using sterile filter paper and funnel.

Preparation of Mcfarland standard

The turbidity standard equivalent to 0.5 McFarland standards was prepared by adding 1ml of concentrated tetraoxosulphate (VI) acid to 99ml of water. 0.5g of dehydrate barium chloride was dissolved in 50ml of distilled water. After which, 0.6ml of the barium chloride solution was added to 99.4ml of the acid solution. The mixture was mixed properly and a small portion of the turbid solution was transferred into a corked tube and stored properly at room temperature until use.

Preparation of nutrient agar

Nutrient agar sterilized in a flask in the autoclave at 121°C for 15 mins at 15 pounds per square inch (psi) and cooled to 40 - 50°C was poured aseptically, in the volume of 20ml each, into sterilized Petri dishes and allowed to harden under room temperature. The plates were placed in the incubator for 24hrs at 37°C to check for its sterility prior to antimicrobial test.

Determination of antimicrobial activity of plant extracts by Agar well diffusion

The test organisms (*S. aureus* and *E. coli*) were each streaked or spread on the surface of the prepared nutrient agar medium. The inoculated plates were allowed for 30mins at room temperature for the organisms to pre-diffuse. After which, the inoculated plates were punched with a 5mm cork borer to make wells in the agar plate. The wells were each filled with the different plant extracts aseptically and after which, the plates were incubated at 37°C for 24hrs. The antibacterial activity of the active constituents of the plant extracts on each of the test organism were determined by measuring inhibition zone diameter (IZD) in millimeters (mm), caused by these plant extracts on the organism, using a calibrated meter rule

Results And Discussion

The cold water extract of *A. indica* showed no antibacterial activity while the hot water and ethanolic extracts of *A. indica* showed maximal antibacterial activity with inhibition zone diameters of ethanolic extract at 27mm for *S. aureus* and 40mm for *E. coli* while hot water extract at 18mm/ml showed 30mm for

E. coli and 27mm for *S. aureus*. (Table 1). This findings partly corroborated with a study conducted by Aromdee *et al* (2006) and Mamman *et al* (2013) who showed that the crude extract of *A. indica* showed antibacterial activity against *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The difference in the effect of this plant extracts within the organisms suggested that there are different antibacterial compounds in the plant extracts and that the compound that acted on one may not be the same as the one that acted on the others since antibacterial agents have different modes of action (Aliu, 2007). This phenomenon of varied susceptibility was also observed by Ergene *et al* (2006). The kill-time of both the ethanol and aqueous extracts of *A. indica* on Gram-negative organisms was much longer than on Gram-positive organisms. This might be due to the more complex nature of the cell wall of Gram negative organisms as compared with Gram positive organisms. The cell wall of Gram-positive organisms is single-layered; while that of Gram-negative bacteria is multilayered and also

bound by an outer cell membrane (Yoa and Moellering, 1995). In corroboration to this finding; Jahan *et al* (2007) showed 92% susceptibility of *E. coli* and *S. aureus* to petroleum extract of *A. indica* oil.

Fresh ethanolic extracts of *A. sativa* at 0.5mg/ml showed 28mm as maximal IZD antibacterial activity against the test bacteria while the fresh extract of *Z. officinale* showed 18mm maximal IZD antibacterial activity against *S. aureus* but no IZD against *E. coli* (Table 2). This corresponds to study by Ushimaru *et al* (2007) who showed that the ethanolic extracts of *A. sativum* showed antibacterial activity against *E. coli* and *S. aureus*. Though our study used only the fresh extract of *A. sativum*.

The fresh extract of *Z. officinale* (Ginger) exhibited only but little zone of inhibition against *S. aureus* and *E. coli*; with about 70% of the tested organism showing resistance to the FEZO (Table 3). In relation to this, Ushimaru *et al* (2007) showed that the ethanolic extract of *Z. officinale* (Ginger) showed antibacterial activity against *E. coli* and *S. aureus*.

Table 1: Antibacterial activity of *A. indica* (neem) in form of inhibition zones (mm) on test bacteria

Extract concentration (mg/ml)	Inhibition Zone Diameter (mm)							
	Aqueous ethanolic extracts		Cold water extract		Hot water extract		Ampicillin	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
2	15	14	-	-	-	-	-	-
4	-	24	-	-	-	13	-	-
6	15	26	-	-	-	15	-	-
8	16	30	-	-	12	14	-	-
10	13	31	-	-	14	18	-	-
12	25	34	-	-	14	19	-	-
14	30	34	-	-	18	23	-	-
16	30	36	-	-	20	27	10	-
18	27	40	-	-	20	30	12	-

Key:

- = No inhibition

S. aureus = *Staphylococcus aureus*

E. coli = *Escherichia coli*

A. indica = *Azadirachta indica*

A. Sativum = *Allium sativum*

Z. officinale = *Zingiber officinale*

A. cepa = *Allium cepa*

Table 2. Inhibition zone diameters (mm) of fresh *Allium sativum* (Garlic) and *Z. officinale* (Ginger) on test bacteria

Extract concentration (mg/ml)	IZD on FEAS		IZD on FEZO		IZD on Ampicillin	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
0.1	25	-	8	-	-	-
0.2	24	16	10	-	-	-
0.3	22	22	11	-	-	-
0.4	20	24	14	-	-	-
0.5	28	28	18	-	-	-

Table 3. Inhibition zone diameter of fresh extracts of *Allium cepa* (onion) on test bacteria

Extract concentration (mg/ml) of <i>A. cepa</i>	IZD of FEAC (mm)		Extract concentration of Ampicillin	IZD (mm) on Ampicillin	
	<i>S. aureus</i>	<i>E. coli</i>		<i>S. aureus</i>	<i>E. coli</i>
0.1	-	-	2	-	-
0.2	-	-	4	-	-
0.3	-	-	6	-	-
0.4	-	-	8	-	-
Raw	-	-	10	-	-

Key:

- = No Inhibition

FEAC = Fresh extract of *Allium cepa*FEAS = Fresh ethanolic extract of *Allium sativum*FEZO = Fresh ethanolic extract of *Zingiber officinale*

The fresh ethanolic extract and aqueous (cold water and hot water) of *A. cepa* (onions) produced no antibacterial activity against the tested bacteria at all concentrations but raw extract of Ginger showed maximal IZD against *S. aureus* at 10mm but no activity against *E. coli* at all concentration. This study was partly in consonance with study by Islam *et al* (2014) who showed the potent antimicrobial activity of the ginger extract against the all tested bacterial pathogens. Soybean oil extract of ginger showed highest zone of inhibition (11.67±1.53mm) against *Salmonella* spp. and lowest zone of inhibition (8.0±1.73mm) against *Escherichia coli*. Ginger extract also showed lower zone of inhibition (8.67±2.52mm) against *Staphylococcus aureus* compared to the Gram-negative bacteria. Ginger was active against *S. aureus* probably because it is a cell wall inhibitor. The test bacteria were resistant to onion extracts, this did not corroborate with study by Azu and Onyeagba (2006), who revealed that ethanolic extract of ginger gave the widest zone of inhibition against two out of the three test organisms at the concentration of 0.8gml⁻¹. However, *Escherichia coli* and *Salmonella typhi* were more sensitive to the extract of onion bulbs compared to *Bacillus subtilis* which was predominantly resistant. The factors responsible for this high susceptibility of *Escherichia coli* and *Salmonella typhi* to the extracts are not exactly known but may be attributed to the presence of secondary plant metabolites (Nweze *et al.*, 2004). In the antibiotic sensitivity tests, all the isolates were resistant to ampicillin. The resistance of the bacterial isolates to this commonly used antibiotic could be attributed to indiscriminate and irrational use of these drugs in humans which usually results in persistent resistance developed by these microbes.

Conclusion

This study has demonstrated the potential use two (*A. indica* and *A. sativum*) tropical plants as an antibacterial agent for the treatment of abscess caused by *S. aureus* and *E. coli*. Despite the fact that other plants used in this study showed no antibacterial activity, they can be analyzed further to know their efficacy against other pathogens. More so, those that showed antibacterial activity should be subjected to further analysis in order to unravel the active ingredients that caused effects on the growth of these two organism.

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