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Antimicrobial properties of the extracts of locally sold garlic and neem leaf in Benin City, Nigeria

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Abstract

Different varieties of medicinal plants have been reported and a recent study on various herbal plants sold in Benin City, Nigeria has revealed the presence of disease causing organisms present on them. This report questions the antimicrobial potency of these locally sold varieties of medicinal plants. In this study, we obtained garlic and neem leaf from local herbal practitioners in Benin City, Nigeria and prepared three different extracts (methanol, acetone and chloroform) of both samples. We then used the disc diffusion method of antimicrobial assay to analyse the antimicrobial properties of these extracts against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi*. Our results showed that at least two of the test organisms are susceptible to each garlic extract, with the chloroform extract having the most antimicrobial property against all microorganisms at concentration as low as 2.26mg/ml. The extracts of neem leaf however showed antimicrobial property against *S. aureus* and *E. coli* only with the acetone extract having the highest antimicrobial potency against these two organisms at a concentration of 6.84mg/ml. These results thus revealed the antimicrobial property of locally sold garlic and neem leaf for herbal medicines. However, it is suggested that these plants be thoroughly washed with water before use to reduce or eliminate any microbial contamination due to field contamination.

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Introduction

The use of natural products from plants in the treatment and control of microbial infections is doubtlessly as old as man itself. Since the first documentation of the use of phytomedicines was made in 2800 BC, there has been a tremendous increase in the use of plants for medicine. Today, over 80% of the world's population resort to one form of phytomedicine or the other as their first line of treatment for various diseases (Pandy *et al*., 2011). Various parts of plants have been shown to have remarkable lethal effect on both prokaryotic and eukaryotic organisms and therefore they have been employed in the treatment of diseases caused by such organisms (Joshi *et al*., 2011; Shobana *et al*., 2009). The emergence of 'wonder drugs' or antimicrobial chemotherapy in the 1930s following the discovery of Gerhard Domagk, a German pharmacologist who out of desperation used a dye meant for tinting cloths to cure his sick daughter of streptococcal infection, brought about a rapid decline in phytomedicines (Okonko *et al*., 2008). However, the development of antimicrobial resistance in the 1970s (Bisht *et al*., 2009) coupled with the high cost of chemotherapeutic drugs led to the search for alternative cure to drug resistant microbes. These synthetic drugs also brought along severe side effects. According to Joshi *et al*. (2011), over 2.2 million people were hospitalized in a single year in the United States as a result of adverse drug reactions which also led to about 106,000 deaths. These incidences triggered the resurgence of phytomedicines about a few decades later.

In Nigeria, over 80% of the Country's population depend on one form of phytomedicine or the other (Okigbo and Nmeka, 2006) with it been the major source of treatment for those living in the rural communities. In recognition of the beneficial effects of these herbal drugs, various state governments are setting up traditional medicine board in the Country while the National Agency for Food, Drug Administration and Control (NAFDAC) has started the registration of herbal products for use in the treatment of various forms of infections and diseases (Abere *et al*., 2007). Garlic and neem are two major plants that are employed in the treatment of several diseases and microbial infections in Nigeria. Various parts of neem plant and garlic bulb have been investigated for their antimicrobial properties by different authors (Onyeagba *et al*., 2004; Widowati *et al*., 2007).

The different parts of neem plant such as its leaf, root and back as well as the bulb of garlic are sold in local market by herbal practitioners and market women who bring these products from the rural communities to urban areas for sale to people suffering from one form of ailment or the other. The lack of scientific evidence to back up the potency and efficacy of these locally sold garlic bulb, neem leaf and other herbal products is a major limitation to their wide spread application especially as recent publication has indicated the presence of disease causing organisms in these herbal products, thus labelling them as agents of disease transmission rather than cure (Idu *et al*., 2011).

This study therefore is aimed at analysing the antimicrobial potential of various extracts of garlic and neem leaf obtained from local herbal practitioners on *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium* and *Escherichia coli*.

Materials and methods

Collection of samples and test organisms

Bulb of garlic (*Allium Sativum*) and fresh leaves of neem (*Azadirachta indica*) were obtained from local sellers of herbal products in Benin city Nigeria. They were identified by the Botany Department of The University of Benin, Nigeria. The test organisms *E. coli* K12, *S. aureus* N315, *S. typhimurium* 14028 and *P. aeruginosa* ATCC 27853 were obtained from the veterinary research institute of Nigeria, Vom in Agar slants and maintained at 4^{o}C in the refrigerator for 48 hours before use.

Preparation of extracts

Garlic bulbs were peeled washed with distilled water and then sun dried for three days along with freshly washed neem leaves. The dried cloves and leaves were separately mashed into paste and powder using a laboratory mortar and pestle and a blender respectively. Two hundred and fifty millilitre of each solvents (chloroform, acetone and methanol) were introduced into five hundred millilitre flasks and 80g of garlic paste was added into each solvent while 100g of blended neem leaf powder was also introduced into separate flasks each containing 250ml of the solvents. The mixtures were agitated overnight in a multifunctional oscillator at 120rpm after which the supernatants were separated from the residue by decanting and filtered using a buchnner funnel. The extracts of the neem leaves were passed through activated charcoal contained in a thimble filter to remove any chlorophyll present. The samples were then evaporated to remove traces of the extraction solvent with the aid of a rotary evaporator at 25°C. Each extract solution was evaporated to dryness in a 100ml beaker using an electric thermostatic drying oven at 30°C. The weight of the dry mass were determined and used to calculate the concentration of the extracts in each solution in mg/ml. Extracts were then dissolved in 20ml of sterile distilled water and 100µl of the extracts were individually spread on McConkey agar plate and incubated overnight to test the sterility of the extracts. No growth was observed on overnight cultures of McConkey agar plates indicating that the extracts are sterile. Extracts were kept in the refrigerator at -4°C for use.

Determination of antimicrobial properties

Antimicrobial test were carried out using the disc diffusion method of antimicrobial assay (Bala *et al*., 2005). 5mm diameter discs were made from Watman no. 1 filter paper and sterilized in a glass beaker by autoclaving at 121°C for 15 minutes. Discs were then impregnated in different concentrations of each extract solution and aseptically placed in a MacConkey agar plate inoculated with respective bacteria pathogen. The plates were incubated for 24 hours at 37°C and the inhibition zones were measured using a standard ruler. Extracts with inhibition zones below 7mm were recorded as having no antimicrobial property (ND). Each experiment was carried out in triplicate for statistical accuracy and the average data are presented in the result section.

Results

In order to ascertain the antimicrobial activity of locally sold garlic and neem leaf in Benin City, Nigeria, the antimicrobial properties of their extracts using various solvents were tested by disc diffusion method of antimicrobial assay. Results obtained for the antimicrobial studies of garlic and neem leaf extracts are presented in Table 1 and Table 2 respectively. Of the three extracts of garlic (Chloroform, ethanol and methanol), chloroform extracts had the better antimicrobial property inhibiting the growth of all bacteria at concentrations from 4.53mg/ml for *E. coli* and 2.26mg/ml for *S.aureus*, *P. aeruginosa*, and *S. typhi* (Table 1). Acetone garlic extracts had antimicrobial activity against 2 of the test microorganisms used (*S. aureus* and *S. typhi*) at concentrations from 20.73mg/ml while *E. coli* and *S. aureus* were susceptible to the methanol extract of garlic at concentrations of 4.60mg/ml and above. These results shows the broad antimicrobial property of garlic as all gram negative and gram positive bacteria used in this study were inhibited by the extracts of garlic.

Compared to the extracts of garlic which had antimicrobial activity against all test organisms used, only two of the test organisms (*E. coli* and *S. aureus*) were sensitive to the extracts of neem leaf. The acetone extract of neem leaf was active against *E. coli* and *S. aureus* at concentrations from 6.84mg/ml while methanol extract was active against *S. aureus* at concentrations of 8.84mg/ml and above. The chloroform extract was also active against *S. aureus* at

concentration of 12mg/ml and above while *E. coli* was sensitive to chloroform neem leaf extract only at a concentration of 30mg/ml. Increase in the concentration of the chloroform extract of neem leaf to 30mg/ml had little effect on the antimicrobial properties of the extract (Table 2). Although neem leaf exhibited antimicrobial property on just two of the test organisms, it however demonstrated a broad spectrum of antimicrobial property as it was able to inhibit both the gram positive (*S. aureus*) and a gram negative bacteria (*E. coli*) used in this study.

Table 1. The diameter of the zones of inhibition of garlic extracts at different concentrations.

Garlic extracts	Concentrations (mg/ml)	DIAMETER OF INHIBITION ZONES (mm) *Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeruginosa*
Acetone	0.00	ND	ND	ND	ND
	5.18	ND	ND	ND	ND
	10.36	ND	7.00	ND	ND
	15.54	ND	6.00	ND	ND
	20.73	ND	7.00	12.00	ND
	25.91	ND	14.00	13.00	ND
Chloroform	0.00	ND	ND	ND	ND
	2.26	6.00	19.00	10.00	8.00
	4.53	7.00	25.00	11.00	11.00
	11.32	35.00	27.00	13.00	33.00
Methanol	0.00	ND	ND	ND	ND
	4.60	6.00	8.00	ND	ND
	9.20	5.00	10.00	ND	ND
	13.80	5.00	11.00	ND	ND
	18.18	7.00	16.00	ND	ND
	22.98	9.00	9.00	ND	ND

on the tests organisms at an incubation temperature of 37°C, ND: no antimicrobial activity detected

Table 2. The diameter of the zones of inhibition of neem leaf extracts at different concentrations on the tests organisms at an incubation temperature of 37°C.

Neem extracts	Concentrations (mg/ml)	DIAMETER OF INHIBITION ZONES (mm) *Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeruginosa*
Acetone	0.00	ND	ND	ND	ND
	6.84	7.0	8.0	ND	ND
	13.69	8.0	9.0	ND	ND
	20.54	8.0	10.0	ND	ND
	27.38	8.0	11.0	ND	ND
	34.23	8.0	9.0	ND	ND
Chloroform	0.00	ND	ND	ND	ND
	6.0	6.0	6.0	ND	ND
	12.0	ND	11.0	ND	ND
	18.0	ND	6.0	ND	ND
	24.0	ND	11.0	ND	ND
	30.0	10.0	13.0	ND	ND
Methanol	0.00	ND	ND	ND	ND
	4.24	ND	6.0	ND	ND
	8.48	ND	7.0	ND	ND
	12.73	ND	9.0	ND	ND
	16.97	ND	9.0	ND	ND
	21.21	ND	10.0	ND	ND

ND: no antimicrobial activity detected

Discussion

Our results showed that garlic exhibited a broad spectrum of antimicrobial activity inhibiting all gram negative and the gram positive bacteria used in this experiment. The MIC vary significantly for each extracts with chloroform extracts having the lowest MIC value for all test organisms which range from 2.26mg/ml to 4.53mg/ml. Methanol extracts had an MIC of 4.60mg/ml while Acetone extracts had MIC of 20.73mg/ml for susceptible organisms. These results are similar to those of previous research on garlic antimicrobial properties. Aqueous extracts of garlic were shown to inhibit both gram positive and gram negative bacteria with an MIC of 7 to 21mg/ml for gram positive bacteria and 6 to 11mg/ml for gram negative bacteria (Durairaj *et al*., 2009) while Daka (2011) in their study showed the antimicrobial activity of aqueous garlic extract against *S. aureus* with an MIC of 7.50mg/ml. The low MIC value obtained in this research for chloroform and methanol extracts compared to the acetone extracts as well the aqueous garlic extracts reported in previous research is likely a result of the chemical complexity of garlic (Harunobu, 2006).

As a result of garlic's chemical complexity, various solvent extract different bioactive materials. These results therefore explains that the chloroform extracts of locally sold garlic contains bioactive compounds which are more effective against the test organisms used in this experiment compared to acetone and methanol extract as it showed antimicrobial properties against all test organisms at much lower concentrations compared to the other extracts. *S. typhi* was found to be resistance to the methanol extracts of garlic but susceptible to both the chloroform and acetone extracts. Similar results were reported by Bakht *et al*. (2011).

Neem leaf also showed a broad spectrum of antimicrobial activity from the results obtained in this study as its extracts successfully inhibited the growth of the gram positive organism *S. aureus* and one of the gram negative bacteria (*E. coli*) used in this experiment. The acetone extracts showed the most antimicrobial activity against *S. aureus* and *E. coli* with an MIC of 6.84mg/ml while the chloroform extract was least active against *S. aureus* and *E. coli*. The antimicrobial potency of ethanol extracts of neem leaf were recently reported against *S. typhi* by Joshi *et al*. (2011). Contrary to the result obtained in this experiment, Joshi *et al*., (2011) in their study, showed that extracts of neem leaf were active against *P. aeruginosa* and *S. typhi* but resistance to *E. coli*. The differences in the antimicrobial characteristics exhibited by the different solvent extracts of neem leaf reported in this experiment and that by Joshi *et al*. (2011) is likely a result of the solvent used as well as differences in extraction procedures both of which have been highlighted as factors that affects the quality or efficacy of the extracts of medicinal plants (Tiwara *et al*., 2011, Turkmen *et al*., 2007) Koul *et al*., (2009) studied the potential use the extracts of neem leaf as a treatment for inflammations caused by *S. typhi* infection and reported that the petroleum ether and aqueous extracts of neem leaf significantly reduced inflammations caused by *S. aureus* and their associated proteins. Similarly, Biswas *et al*. (2002) successfully applied extracts of neem leaf in the treatment of various skin infections of which *S. aureus* is recognised as a major causative agent of skin infections.

Conclusion

The presence of different strains of disease causing microorganisms on locally sold medicinal plants in Benin City, Nigeria has questioned the antimicrobial efficacy of these plants and implicated them as agents of disease transfer rather than cure. From this study, we have shown the susceptibility of different disease causing organisms including *E. coli, S. aureus, P. aeruginosa* and *S. typhi* to various extracts of garlic and neem leaf thus eliminating public fears on the antimicrobial potency of these plants. It is however

advised that these plants be watched thoroughly with clean water before use to eliminate surface contamination due to field microbial contamination.

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