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Comparative Study of Genetic and Antibacterial Profiles of Nigerian Indigenous and Exotic Varieties of Garlic (Allium sativum)

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Abstract

Extracts and isolated compounds of Allium sativum (garlic) have been found to be of health benefit. The study was aimed at assessing the effects of crude garlic extracts on urinopathogens of pregnant women, as well as to compare the antibacterial and genetic profiles of Nigerian indigenous and exotic varieties of garlic. Biodata and urine samples were collected from two hundred (200) healthy pregnant women attending antenatal clinics. The urine samples were subjected to urinalysis and bacteriological investigations. The subjects were 20 - 43 (31.03 ± 1.46) years old, with modal age 25-30 years. Urinalysis of subjects' urine samples showed no nitrituria, haematuria and bilirubinuria. However, glucosuria (1.5%), ketonuria (3%), leukocyturia (15%) and proteinuria (24%) were detected. Bacterial loads of the urine samples range from 0 to 1100 (with mean value of 315.72) CFU/mL, an indication of non-urinary tract infection bacteriuria. Bacteria isolated from the urine samples were: Escherichia coli, Klebsiella pneumoniae, Klebsiella variicola, Enterobacter cloacae, Pseudomonas aeruginosa, Proteus mirabilis, Citrobacter freundii, Corynebacterium accolens, Actinomyces urogenitalis, Luteococcus sanguinis and Bacillus cereus among others. The bacterial isolates showed high prevalence of multidrug resistant bacteria, with resistance to 2-8 drugs. The filtrates of crushed and centrifuged bulbs of both the indigenous and exotic varieties of garlic produced high antibacterial activities, while both ethanolic and methanolic extracts of garlic did not produce antibacterial activity. The indigenous variety showed higher antibacterial activities and protein qualities than the exotic variety, with both varieties showing genetic diversity. In conclusion, the Nigerian indigenous garlic was found to be of high antibacterial and protein qualities; and for maximal health benefit garlic needs to be chewed or crushed and consumed directly.

Keywords: *Allium sativum, Bacteria, Drug resistance, Pregnancy and Urinary tract infection.*

INTRODUCTION

Garlic (*Allium sativum*) is a species in the onion genus *Allium* and Family Amaryllidaceae (Batiha *et al*., 2020). The garlic bulb is made up of 65 % water, 28 % carbohydrate, 2 % protein, 2.3 % organosulfur compounds and 1.5 % digestive fibre. The organosulfur compounds are the main bioactive compounds and are attributable to all the known health benefits of garlic. These organosulfur compounds found in garlic are allicin, vinyldithiins, ajoenes and diallylpolysulfides. When garlic is chewed, sliced or crushed, it releases an alliin metabolite allicin, which turns into a variety of volatile fat and water soluble sulfur–containing compounds. Allicin exhibits antimicrobial activities against both Gram-positive and Gram-negative bacteria. Garlic has greater antimicrobial activity than other *Allium* because of its richness in allicin (Nakamoto *et al*., 2020). Extracts and isolated compounds of *Allium sativum* have been found to be of potent biological activities including antibacterial, antiviral, antifungal, antiprotozoal, antioxidant, anti-inflammatory and anticancer activities among others (Batiha *et al*., 2020). Human urine can inhabit bacterial growth due to its favourable chemical composition (Acharya and Jadav, 1980). Urinary tract infection (UTI) entails microbial growth within the urinary tract i.e. anywhere from the kidney, the ureter, the bladder and through to the urethra. In all surveys that have been carried out (except in newborns), females are more prone to urinary tract infection than males (Mbakwem-Aniebo and Ene, 2006; Habak and Griggs, 2020). The anatomy of the urethra in men prevents infection: the long urethral length (20 cm) gives a distant barrier

which excludes microorganisms from the bladder. In contrast, the short length of the female urethra (5 cm) is an important factor in the prevalence of urinary tract infection in women (Steven, 2020).

Pregnancy increases the risk of urinary tract infection, at around the 6th week of pregnancy, due to the physiological changes of pregnancy. The ureters begin to dilate (known as hydronephrosis of pregnancy) due to obstruction in the flow of urine, which peaks at 22-26 weeks and continues to persist until delivery. Both progesterone and oestrogen levels increase during pregnancy and these results in decreased ureteral and bladder tones. All these factors combined, leads to urinary stasis and ureterovesical reflux (Delzell and Lefevre, 2000). Also, during pregnancy the body immunity is reduced and this appears to encourage the growth of both commensal and non-commensal microorganisms. The physiological increase in plasma volume during pregnancy decreases urine concentration and up to 70% pregnant women develop glucosuria, a condition in which glucose is discharged in the urine; diabetes mellitus, which encourages bacterial growth in urine (Lucas and Cunningham, 1993). Urinary tract infection during pregnancy contributes significantly to maternal and perinatal morbidity, abortion; low birth weight, maternal anaemia, hypertension, preterm labour, phlebitis, thrombosis and chronic pyelonephritis. (Akerele *et al*., 2001).

The study was aimed at determining the effects of garlic extracts on bacterial isolates from urine of pregnant women, as well as to compare the antimicrobial and genetic profiles of the two varieties of garlic available in Nigerian market.

MATERIALS AND METHODS

Study area and population

This study was carried out at the ante-natal clinics of Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti, Nigeria between 2014 and 2017. Approval was sought and collected from the Research/Ethics Committee of the Ekiti State University Teaching Hospital before the commencement of the research. Two hundred (200) pregnant women attending the antenatal clinics of the hospital were enlisted into the study upon informed consent. Demographic information such as age, occupation, parity, gestational age, previous history of UTI, educational status and sexual activity during pregnancy were obtained from the pregnant women using standard questionnaires.

Sampling Technique

Mid-stream urine specimens were self-collected into sterile universal containers by the eligible pregnant women at EKSUTH. All specimens were transported from the hospital to Microbiology Laboratory of Afe Babalola University, Ado-Ekiti, Nigeria in cold box for culture and microscopy.

Urine microscopy

Ten millilitre of each well mixed urine sample was centrifuged at 1000g for 5min, the supernatant was discarded and a drop of the well mixed sediment was transferred unto a microscope slide and covered with a cover glass before it was examined microscopically at high magnification for pus cells, red blood cells, epithelial cells, casts, crystals and yeast cells.

Urinalysis

Using a Combi-10 strip, biochemical analysis was carried out on the urine samples for the presence of protein, nitrite, glucose, blood, leukocyte esterase, bilirubin, urobilinogen, specific gravity, pH and ketones; where a positive result of protein, nitrite and leukocyte esterase were considered significant to indicate a urinary tract infection.

Bacteriological analysis

Using a micropipette, 0.1mL of well-mixed un-centrifuged urine was inoculated on plate count agar and Cysteine lactose electrolyte deficient (CLED) agar without indicator, using the pour plate method. The plates were incubated aerobically at 37^{o}C for 24 hours and counts were expressed in colony forming units per millimetre (CFU/mL). District colonies were sub cultured severally on nutrient agar plates to obtain pure cultures.

All isolates were characterized using standard bacteriological and biochemical tests, as described by Barrow and Feltham (1993) and identified with the help of online Gideon Informatics (1994-2020).

Antibiotic susceptibility test

All the isolated organisms were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method on Mueller-Hinton agar. This was carried out by making an even spread of the pure isolates on prepared Mueller-Hinton agar using sterile swab sticks and aseptic placement of the antibiotic discs using sterile forceps. The plates were incubated aerobically at 37ºC for 24 hours after which the zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2018). Antibiotics used were augmentin (amoxycillin/clavulanic acid), AUG (30µg); ofloxacin, OFL (5µg); gentamicin, GEN (10µg); nalidixic acid, NAL (30µg); nitrofurantoin, NIT (200µg); amoxycillin, AMX (25µg); tetracycline, TET (25µg) for Gram negative isolates; and augmentin, AUG (30µg); co-trimoxazole, COT (25µg); cloxacillin, CLX (5µg); erythromycin, ERY (5µg); gentamicin, GEN (10µg);

streptomycin, STR (10µg); tetracycline, TET (10µg); and chloramphenicol, CHL (10µg) for Gram positive isolates.

Plant sample collection

Two types of garlic bulbs (*Allium sativum*) were used in this study, the indigenous type and the exotic garlic type (Plate 1). Both the indigenous and exotic garlic bulbs were purchased from the 'Oja-Oba' market in Ado-Ekiti. The indigenous garlic was cultivated in Nigeria while the exotic was imported from South Africa.

Plate 1: Indigenous and exotic garlic available in Nigeria market.

Preparation of Garlic extracts

Fresh garlic bulbs were separated from the cloves and peeled to obtain the edible portion; 50 g of the edible portion were washed thoroughly with distilled water, chopped into pieces and crushed with mortar and pestle, it was then centrifuged, the supernatant was separated and the crude garlic extract was sterilized by membrane filtration.

Also garlic bulbs were chopped into pieces, air-dried and ground into fine powder. Solvent extractions were carried out by mixing 100 g each of the powdered garlic into 400 mL of ethanol and methanol respectively for 72 hours. Filtration with Whatman® filter paper was carried out, followed by removal of theextractants from the filtrate to obtain semi-solid extracts with the aid of water bath at 40°C.

Antimicrobial activity of Garlic extracts using Agar-well diffusion method

Susceptibility of the isolated bacteria to garlic extracts was determined by agar well diffusion technique using Mueller-Hinton agar. Seven millimeter (7mm) diameter wells were prepared on Mueller-Hinton agar containing a suspension of each isolated organisms. Two-fold dilutions of the crude garlic extracts (sap), as well as varied concentrations of ethanolic and methanolic extracts of garlic were added to the wells. The plates were left at ambient temperature for 15min and then incubated at 37ºC for 24hr, after which the zones of inhibition were observed and recorded.

Comparative Protein Ultraviolet (UV) Absorption Spectra Analysis of the Garlic varieties

A total of 10 indigenous and 10 exotic garlic cloves were used for protein extraction. The protein extraction procedure used was as described by Barbarino and Lourenco (2005). Five (5) grams of each of the two garlic varieties were separately grinded in 10 mL of 0.1M NaOH at room temperature using mortar and pestle; the homogenate was transferred into 2.5 mL Eppendorf® tubes and incubated at room temperature for 12 hr. Following, incubation, the homogenate was centrifuged at 4°C at 12,000rpm for 20min using refrigerated centrifuge. The supernatant was collected and stored at 4ºC for UV absorbance spectrum analysis. The procedure used for UV absorption spectrum analysis of protein extracts and protein standards (bovine serum albumin) was as described by Mattley and Garcia-Rubio (2001), where 0.4 mL of the protein extracts were added to 1.6 mL of 0.1M NaOH, mixed well, and the optical density (OD) and absorbance (A) values were measured from A200 to A960 UV wavelengths using Spectronic 20®. Also, 0.4mL each of 5, 10, 15, 20 and 25 mg/mL of protein standard were added to 1.6mL of 0.1M NaOH, mixed well, and the optical density (OD) and absorbance (A) value was taken from A200 to A960 UV wavelengths using Spectronic 20®.

Genomic DNA extraction

The genomic DNA of the garlic bulbs were extracted by the CTAB technique (Doyle and Doyle, 1987). Garlic tissues were cut and blended in 600 µL of extraction buffer and incubated at 65°C for 20 min. The samples were removed from the incubator and allowed to cool to room temperature, followed by addition ofchloroform and gentle shaking to mix. Thereafter, the samples were spun at 14,000 rpm for 15 min and the supernatant transferred into a new Eppendorf® tube and equal volume of cold isopropanol added to precipitate the DNA. The samples were kept in the freezer for 1hr and later spun at 14,000 rpm for 10 min; the supernatants were discarded and the pellets washed with 70 % ethanol and air dried for 30 min on the bench. The pellets were then re-suspended in 100 µL of sterile distilled water. DNA concentrations of all the samples were measured using spectrophotometer at 260 nm and 280 nm and the genomic purity determined. The genomic DNA was used in PCR amplification using RAPD markers

DNA Electrophoresis

Agarose gel electrophoresis was equally used to evaluate the quality and integrity of the DNA by size fractionation on 1.0 % agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose

in 100mL 0.5X TBE buffer solution. The gels were allowed to cool down to about 45°C and 10 µL of 5 mg/mL ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 µL of the DNA with 5ul sterile distilled water and 2 µL of 6X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80V for 2 hr. The integrity of the DNA was visualized and photographed on UV light source.

Dilution of DNA for PCR

About 10 µL of each DNA was taken into Eppendorf® tube and 990µL sterile distilled water was added to make 1000 µL with the final concentration becoming 20-50 ng/µL.

PCR reaction mix

The reaction mix was carried out in 20µL final volume containing 60– 80ng genomic DNA, 0.1 µM of the primers, 2 mM $MgCl_2$, 125 µM of each dNTP and 1 unit of Taq DNA polymerase. The thermocycler profiles has an initial denaturation temperature of 3 min at 94°C, followed by 45 cycles of denaturation temperature of 94°C for 20 seconds, annealing temperature of 37°C for 40 s and primer extension temperature of 72°C for 40 s, followed by final extension temperature at 72°C for 5 min.

Gel electrophoresis of PCR products

PCR product electrophoresis was carried out by size fractionation in 1.0 % agarose gels. A 200 mL agarose gels (1.0 %) was prepared, allowed to cool down to about 50°C and 10uL of 5mg/mL Ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, the PCR amplicon was loaded in the well created. Electrophoresis was done at 100V for 2 hr. The DNA was visualized and photographed on UV light source.

Samples preparation for SDS-PAGE

Garlic sap (100 µL) was taken into Eppendorf tube and 250 µL of the 0.1 M Tris-HCl pH 7.6 was added, it was later mixed on vortex machine for 1min the spin at 10,000 rpm for 10 min, the supernatants was taken into a fresh tube and kept in fridge for SDS-PAGE.

SDS-PAGE gel electrophoresis

Polyacrylamide gel (12 %), which is separation gel, was prepared and stacking gel of 3% was equally prepared as well. A mixture of 5 µL of the sample extracts (garlic sap) and 5 µL of the loading buffer was boiled at 95°C for 5mins and loaded on the gel. The sample was run on the gel for 60 min at 150 V.

The plate was dismantled and the gel removed, stained in 0.1 M Coomassie blue solution for 1 hr and later distained in several rinses of ethanol/acetic acid solution until the gel became clear for viewing. The picture of the gel was later taken for documentation.

Data Analysis

The banding patterns of DNA electrophoresis and SDS-PAGE were transformed into numerical values, where the presence of a band is scored as 1 and absence of a band was scored as 0. The binary value was transferred into NTSYS software v2.02 for analysis using UPGMA method Rolf *et al.* (2000). Associations between variables were determined with Model Selection Loglinear statistic using SPSS V-20.0.Values $p \leq 0.05$ were considered significant at 2 tailed tests.

RESULTS

The two hundred (200) pregnant women enlisted in the study were 20-43 (31.03 ± 4.46) years old, with modal age 25-30 years. Urinalysis showed that no nitrite, blood and bilirubin found in the urine of the subjects. However, glucosuria (1.5%), ketonuria (3%), leukocyturia (15%) and proteinuria (24%) were detected. Significant associations were found between some of the subject's biodata and biomarkers of infection such as: frequency of urination/age; gestation/leukocyte content of urine; abdominal pain/gestation; abdominal pain/leukocyte content of urine; frequency of urination/protein content of urine; leukocytes and protein concentrations in urine among others (Table 1). Bacterial loads in urine of the pregnant women were 0-1100 (315.72 ± 23.44) cfu/mL. Yeast and epithelial cells were found in 62.9 and 29% of the subjects' urine respectively.

Table 1: Associations between biodata and biomarkers of the antenatal subjects.

Associations	$\chi 2$	p value	df
Age/Urination	27.952	0.002*	10
Specific Gravity/Gestation/Abdominal Pain	17.980	0.006*	6
Specific Gravity/Urination/Gestation	60.311	0.001*	30
Specific Gravity/Gestation/Age	36.040	<0.001*	12
Urination/Protein	18.597	0.045*	10
Gestation/Leukocytes	10.857	0.028*	4
Parity/ Specific Gravity	25.182	0.048*	6
Specific Gravity/pH	37.297	<0.001*	6
Bacteria/Leukocytes	5.948	0.429	6
Bacteria/Protein	5.274	0.153	3
Leukocyte/Protein	6.642	0.036*	2
Abdominal pain/Leukocytes	8.448	0.015*	2
Gestation/Bacteria	6.973	0.323	6
Abdominal pain/Bacteria	4.570	0.206	3
Abdominal pain/Gestation	7.170	0.028*	2

*Significant association between variables; df - Degree of freedom

Bacteria isolated from urine were: *Escherichia coli, Klebsiella pneumoniae, Klebsiella variicola, Enterobacter cloacae, Pseudomonas aeruginosa, Proteus mirabilis, Citrobacter freundii, Serratia marcescens, Chromobacterium violaceum, Acinetobacter baumannii, Corynebacterium accolens, Actinomyces urogenitalis, Luteococcus sanguinis, Branchiibius cervicis, Staphylococcus saprophyticus, Bacillus cereus* and *Staphylococcus aureus* among others (Figure 1).

Figure 1: Frequency of occurrence of bacteria isolated from urine of pregnant women.

The bacterial isolates showed high degree of resistance to the tested antibiotics. The Gram negative bacteria were 100% resistant to augmentin and amoxycillin, but they were highly susceptible to ofloxacin (97.37 %). All the Gram positive bacteria were resistant to augmentin, erythromycin, tetracycline and gentamicin (Table 2). There was a high prevalence of multidrug resistant bacteria, showing resistance to 2-8 drugs (Table 3).

The crude extracts (crushed and squeezed) of garlic from both the local and exotic varieties produced highly potent antimicrobial effects. All the bacterial isolates, with the exception of *Pseudomonas aeruginosa* tested were highly susceptible to the crude extracts of garlic. The indigenous garlic showed higher antibacterial activities than the exotic (Table 4). The ethanolic and methanolic extracts of dried garlic bulbs of both varieties did not produce any antibacterial activity.

The absorbance spectra of the protein standard (bovine serum albumin) produced a single peak between 200-400nm wavelengths with highest absorbance of 2.297 at 240 nm wavelength. There were comparative differences between the absorbance values of protein extracts of garlic and that of the protein standard. The protein extract of indigenous variety of garlic produced a single peak between 200-960 nm wavelengths with highest absorbance of 4.532 at 240 nm wavelength. The absorbance of exotic breed has variable peaks of 4.223 at 200-540 nm; 1.60 at 560-620 nm and 0.06 at 920-960 nm. The indigenous variety gave relatively higher protein absorbance values that the exotic breed (Figures 2 and 3).

Table 2: Antibiotics susceptibility pattern of bacterial isolates from urine of pregnant women.

Antibiotics	Bacterial Susceptibility (%)	
	Gram negative	Gram positive
Amoxycillin/Clavulanic acid (30µg)	0	0
Ofloxacin (5µg)	93.37	-
Gentamicin (10µg)	17.11	0
Nalidixic acid (30µg)	32.89	-
Nitrofurantoin (200µg)	17.11	-
Amoxycillin (25µg)	0	0
Tetracycline (25µg)	11.84	0
Cotrimoxazole (25µg)	17.11	18.70
Cloxacillin (5µg)	-	0
Erythromycin (5µg)	-	0
Streptomycin (10µg)	-	0
Chloramphenicol (10µg)	-	28.13

– Not done

Table 3: Cluster of drug resistance by bacteria isolated from urine of pregnant women.

Clusters of Resistant Drugs	Frequency
Gram Negative	
AUG/TET/AMX/COT/NIT/GEN/NAL/OFL	2
AUG/TET/AMX/COT/NIT/GEN/NAL	23
AUG/TET/AMX/COT/NIT/GEN	3
AUG/TET/AMX/COT/NIT	1
AUG/TET/AMX/COT/GEN/NAL	1
AUG/AMX/COT/GEN/NAL	2
AUG/AMX/GEN	1
AUG/AMX	1
Gram Positive	
AUG/AMX/ERY/TET/CLX/GEN/COT/CHL	13
AUG/AMX/ERY/TET/CLX/GEN/COT	2
AUG/AMX/ERY/TET/CLX/GEN	1

The four primers used (OPT-O6, OPT-11, OPB-14 and OPT-20) provided evaluable bands though, OPT-06 did not amplify any fragment in L1, and as such, OPT-06 was not included in the pooled matrix dendrogram for the RAPD markers. Polymorphic bands were observed with each primer with OPT-20 having the highest polymorphism (71 %) and OPT-14 with the least polymorphism (38 %). OPT-06 and OPT-11 have 57 % polymorphism each. A total of 55 % of the produced markers were polymorphic. OPT-20 produced 5 polymorphic bands, OPT-06 and OPt-11 produced 4 polymorphic bands each while OPB-14 produced 3 polymorphic bands (Figures 4 and 5).The local varieties were grouped in separate clusters, this shows that they are genetically different from the exotic variety (Figure 5). The polymorphism percentage ranged from 38 to 71 %. The SDS-PAGE electrophoregram profile revealed 21 bands of molecular weights ranging from 70 kDa to 16 kDa (Figures 6 and 7).

Table 4: Antimicrobial effects of dilutions of crude garlic extracts (sap) on bacteria isolated from urine.

Organisms	Indigenous garlic sap						Exotic garlic sap					
	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}
Klebsiellapneumoniae	7	3	0	0	0	0	6	3	0	0	0	0
Enterobacter cloacae	13	8	7	4	0	0	14	9	5	0	0	0
Escherichia coli I	15	13	8	3	0	0	15	10	7	2	0	0
Escherichia coli II	18	15	12	6	0	0	17	13	8	0	0	0
Shigella flexneri	15	13	10	8	3	0	17	14	9	7	0	0
Providencia stuartii	14	13	10	6	0	0	17	12	0	0	0	0
Citrobacter freundii I	13	11	8	3	0	0	15	10	6	3	0	0
Citrobacter freundii II	11	7	4	2	0	0	8	6	3	0	0	0
Acinetobacter baumannii	18	13	7	0	0	0	16	10	0	0	0	0
Citrobacter youngae	27	19	17	13	7	0	29	21	18	13	6	0
Proteus vulgaris	24	19	12	6	0	0	21	16	10	6	0	0
Proteus mirabilis	28	24	19	15	8	2	27	23	17	15	5	0
Pseudomonas aeruginosa I	3	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa II	3	0	0	0	0	0	5	0	0	0	0	0
Corynebacterium accolens I	19	16	12	9	0	0	20	15	11	3	0	0
Corynebacterium accolens II	8	5	3	1	0	0	8	4	0	0	0	0
Corynebacterium accolens III	8	3	2	1	0	0	8	4	1	0	0	0
Luteococcus sanguinis	14	13	11	12	4	0	14	12	10	8	0	0
Staphylococcus epidermidis	21	18	15	13	6	2	22	17	16	14	7	0
Staphylococcus aureus	20	18	14	12	5	0	20	16	14	12	2	0
Branchiibius cervicis	17	14	11	10	0	0	18	15	12	10	0	0
Proteus mirabilis ATCC12453	30	25	18	13	5	0	28	23	17	13	8	0
Staphyloccocus aureus ATCC 25923	26	23	20	18	10	3	27	23	21	18	10	2

*Values are zones of inhibition in millimeters (mm)

**Comparing reciprocal of dilution factor at MIC, t=2.381, p=0.033

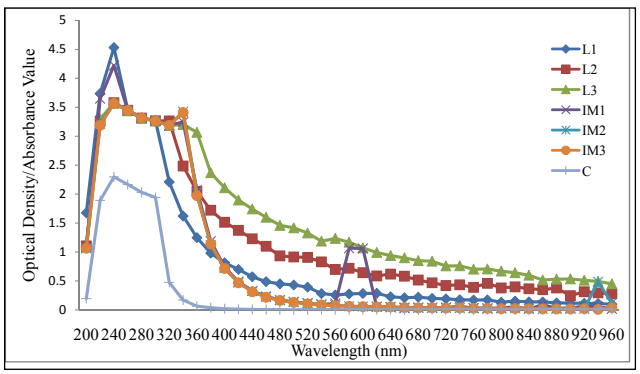

Figure 2: Comparison of absorbance spectra of protein from indigenous and exotic garlic with protein standard (bovine serum albumin)

KEY: L: Indigenous garlic variety; IM: Exotic garlic variety; C: Control (Protein standard- bovine serum albumin)

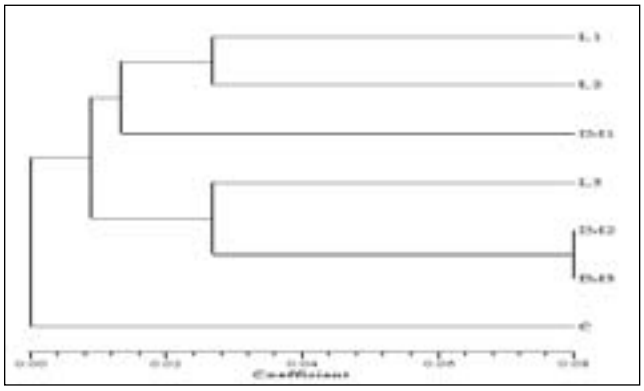

Figure 3: Dendogram of comparison of absorbance spectra of protein from indigenous (L1-3) and exotic garlic (IM1-3) with protein standard (bovine serum albumin)

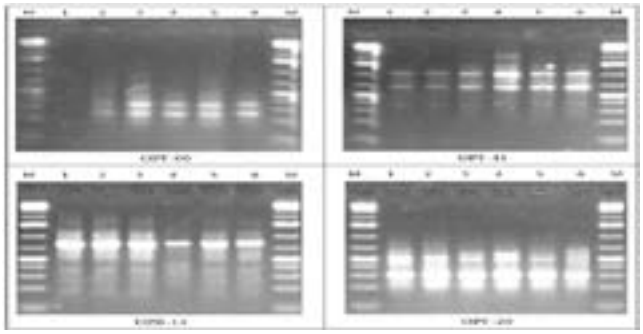

Figure 4: Electrophoresis gel of amplified DNA of indigenous garlic (1-3) and exotic garlic (4-6)

Table 5: Percentage polymorphism obtained from amplified DNA of local and exotic garlic

S\N	Primer Name	Sequences (5' – 3')	Number of Poly-mor-phism	Number of Monomor-phism	Total number of marker	% Polymor-phism
1	OPT-06	CAAGGGCAGA	04	03	07	57
2	OPT-11	TTCCCCGCGA	04	03	07	57
3	OPB-14	TCCGCTCTGG	03	05	08	38
4	OPT-20	GACCAATGCC	05	02	07	71
Total			**16**	**13**	**29**	**55**

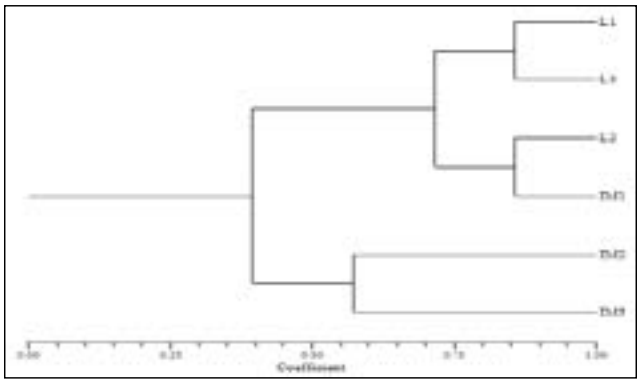

Figure 5: Dendrogram for the RAPD markers for amplified DNA of local garlic (L1 - L3) and exotic garlic (IM1 – IM3)

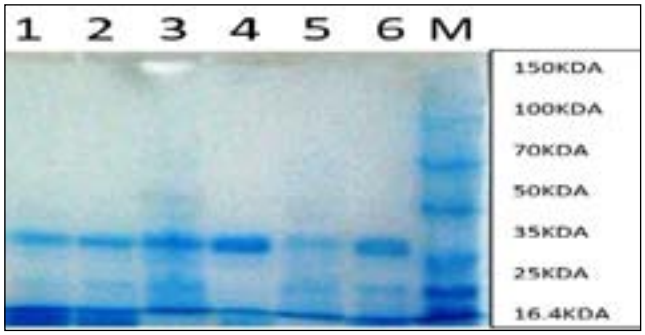

Figure 6: SDS-PAGE of the Garlic samples, local garlic (1 - 3) and exotic garlic (4 - 6)

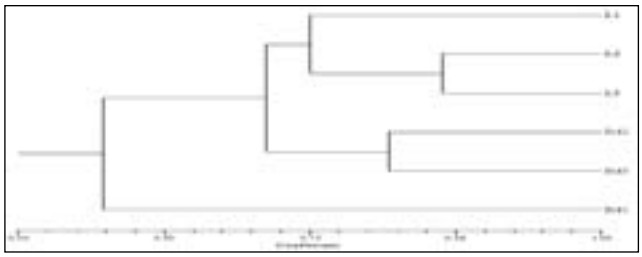

Figure 7: Dendrogram for the SDS-PAGE (local garlic L1- L3; exotic garlic IM1 – IM3)

DISCUSSION

The bacterial profile of urine of pregnant women with asymptomatic bacteriuria was assessed and the antimicrobial effect of garlic against the bacterial isolates determined. All the 200 women studied were healthy, as their visits to the hospital were based on routine antenatal check-ups.

The qualities of the urine samples were within normal with exception of few cases of glucosuria, proteinuria and leukocyturia. The bacteria concentrations in the urine samples were within the normal range. According to Vandepitte *et al*. (2003), bacteria load in urine fewer than 10^4 CFU/mL should be reported as probable absence of UTI. Exceptions are if fewer than 10^4 CFU/mL are present in urine taken directly from the bladder by suprapubic puncture or cystoscopy. Bacteriuria was not significantly associated with any of the biomarkers of UTI and the subjects' biodata. However duration of gestation was significantly associated with abdominal pain, urine specific gravity, frequency of urination, proteinuria and leukocyturia. Significant associations were obtained between proteinuria and leukocyturia; as well as proteinuria and frequency of urination.

The high occurrences of *Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis* and *Pseudomonas aeruginosa* reported in the urine of apparently healthy pregnant women in this study, have been reported earlier studies in urinary tract infection in pregnancy in Nigeria (Abdul and Onile, 2001; Okiki *et al.* 2015; Onukak *et al*., 2021).

The bacteria isolated from urine of pregnant women in present study showed a high degree of resistance to the tested antibiotics. This finding is consistent with the findings of earlier works of Okiki *et al.* (2015) and Asmat *et al.* (2021). However, a significantly higher drug resistant profile was obtained for the urinopathogens reported in present study compared to the antibiotic resistant profiles among urinopathogens reported two decades ago by Akerele, *et al.* (2001). Multidrug resistant bacteria have been emerging worldwide causing diseases in both humans and animals (WHO, 2020).

This study demonstrated high antibacterial potency of garlic extracts against urinary tract isolates, as all the multidrug resistant bacteria were susceptible to the filtrates of crushed and centrifuged garlic bulbs. The local variety of garlic was found to be more potent than the exotic breed. Several studies, including those of Rees *et al.* (1993) and Kumar and Sharma (2002), had previously demonstrated the antibacterial potency of crude garlic extract against pathogens such as *Vibrio parahaemolyticus, E. coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris* and *Staphylococcus aureus*. The antimicrobial potency of garlic has been attributed to its ability to inhibit toxin production and expression of enzymes for pathogenesis (Banerjee *et al.,* 2001). The reconstituted, dried ethanolic and methanolic extracts of garlic in present study did not produce antibacterial activity. This may be attributed to the volatility of organosulfur compounds which are the bioactive constituent of garlic (Abe *et al*., 2020). According to Abe *et al*. (2020), volatile profiles of processed garlic are influenced by processing conditions, such as temperature, pH and solvent. For maximal health benefit, garlic needs to be chewed, crushed and consumed directly.

The primers employed in this study have previously been used successfully in the assessment of variability in different species genotypes. OPT-06 and OPT-20 were used successfully in RAPD profiling and DNA fragmentation examination in brains of Wistar Rats (Ibitayo *et al*., 2017); genetic variability among *Telfaira occidentalis, Celosia argentea* and *Talinum triangulare* (Bello *et al*., 2017). OPT-11 and OPT-20 were used in RAPD marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad (Verma *et al*., 2017). Iqbal *et al*. (2018) have used OPB-14 to assess the genetic diversity in Hybrid Pea Lines (*Pisum sativum* L.).

UPGMA (Unweighted Pair-Group Method using Arithmetic averages) gene cluster analysis based on the Jaccard's similarity coefficient grouped the genotypes into four main clusters; L1; L2 and L3; IM2 and IM3;

and IM1. The local varieties were grouped in separate clusters, this showed that they are genetically different from one another. According to the dendrogram, the L1 and IM1 varieties were distinct from other varieties with 0.75 and 0.58 similarity index respectively. The L2 and L3 showed the highest similarity coefficient at 0.88 while IM2 and IM3 showed close similarity at coefficient of 0.828. So *et al.* (2021) reported the genetic diversity among varieties of garlic in West Africa, which is revealed in their wide variations in colour, shape and number of cloves and the ability to flower.

Also, the electrophoretogram from the SDS-PAGE generated 3 main clusters consisting of 2 varieties of garlic; L1 and L3; L2 and IM1; IM2 and IM3 respectively. The varieties in cluster 1 and 2 showed the highest similarity index of 0.85 each, while the 2 clusters branched at 0.70 similarity index. Cluster 3 has the least index of 0.57 and it branched off from other clusters at 0.40, this shows that cluster 3 (IM2 and IM3) has the most diverse varieties. Electrophoresed purified protein components of garlic have been found to display immunomodulatory and mannose-binding activity (Chandrashekar and Venkatesh, 2009).

CONCLUSION

The result of the present study showed that crude extract of *Allium sativum* (garlic) was effective against multidrug resistant bacteria, with the Nigerian indigenous variety yielding better results.

Conflict of interest: No conflict of interest reported.

Authors' contributions: All authors contributed adequately.

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REFERENCES

Abe, K., Hori, Y. and Myoda, T. (2020). Volatile compounds of fresh and processed garlic. *Experimental and Therapeutic Medicine*, 19(2):1585-593.

Abdul, I.F. and Onile, B.A. (2001). Bacterial isolates from the urine of women in Ilorin and their antibiotic susceptibility patterns. *Tropical Journal of Obstetrics and Gynaecology*, 18(2): 61-65.

Akerele, J., Abhlimen, P. and Okonofua, F. (2001). Prevalence of asymptomatic bacteriuria among pregnant women in Benin City, Nigeria. *British Journal of Obstetrics and Gynaecology*, 221 (2): 141-144.

Asmat, U., Mumtaz, M.Z. and Malik, A. (2021). Rising prevalence of multidrug-resistant uropathogenic bacteria from urinary tract infections in pregnant women. *Journal of Taibah University Medical Sciences*, 6(1):102e111.

Barbario, E. and Lourenço, S.O. (2005). An evaluation of methods for extraction and quantification of protein from marine macro- and micro-algae. *Journal of Applied Phycology*, 17: 447-460

Batiha, G.E., Beshbishy, A.M., Wasef, L.G., Elewa, Y.H.A., Al-Sagan, A.A., Abd-El-Hack, M.E., Taha, A.E., Abd-Elhakim, Y.M. and Devkota, H.P. (2020). Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): a review. *Nutrients*, 12: 872; doi: 10.3390/ nu12030872

Banerjee, S.K., Mukherjee, P.K. and Maulik, S.K. (2001). Garlic as an antioxidant: the good, the bad and the ugly. *Phytotherapy Research,* 17(2): 97–106

Barrow, G.I. and Feltham, R.K.A. (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*. Cambridge University Press, London. 331 pp

Bello, B.K., Adetuyi, F., Oluwaseun, O., Anthonia, O. and Adegoke, S. (2017). Biomass and genetic variability among *Telfaira occidentalis, Celosia argentea* and *Talinum triangulare* harvested from Okitipupa, Nigeria studied by random amplified polymorphic DNA (RAPD). *International Journal of Biosciences and Technology*, 10(5): 30.

Chandrashekar, P.M. and Venkatesh, Y.P. (2009). Identification of the protein components displaying immunomodulatory activity in aged garlic extract. *Journal of Ethnopharmacology,* 124(3): 384-390.

Clinical and Laboratory Standards Institutes, CLSI (2018). *Performance Standard of Antimicrobial Susceptibility Testing*; 28th Information Supplement, CLSI, Wanye, USA.

Delzell, J.E. and Lefevre, M.L. (2000). Urinary tract infection during pregnancy. *American Family Physician,* 61(3):713-21.

Doyle, J.J. and Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19:11-15

Gideon Informatics. (1994-2020). *Gideon Microbiology-Identify Bacteria*. www.gideononline.com.

Habak, P.J. and Griggs, Jr R.P. (2020). Urinary tract infection in pregnancy. In: *Treasure Island* (FL): StatPearls Publishing; Available from: https://www.ncbi.nlm.nih.gov/books/B_K537047

Ibitayo, A.O., Afolabi, O.B., Akinyemi, A.J., Ojiezeh, T.I., Adekoya, K.O. and Ojewunmi, O.O. (2017). RAPD profiling, DNA fragmentation, and histomorphometric examination in brains of Wistar rats exposed to indoor 2.5 GHz Wi-Fi devices

radiation. *BioMed Research International*, 2017: Article ID 8653286, pages.

Iqbal, A., Razzaq, A., Hadi, F., Nisar, M., Ozturk, M. and Altay, V. (2018). Assessment of genetic diversity among hybrid pea lines (*Pisum Sativum* L.) as revealed by random amplified polymorphic DNA (RAPD) markers. *Fresenius Environmental Bulletin*, 27: 6447-6453.

Kumar, A. and Sharma, V.D. (2002). Inhibitory effect of garlic (*Allium sativum* Linn) on enterotoxigenic *Escherichia coli. Indian Journal of Medicine*, 76:66–70.

Lucas, M.J. and Cunningham, F.G. (1993). Urinary tract infection in pregnancy. *Clinical Obstetrics and Gynaecology*, 36:555-568.

Mbakwem-Aniebo, C. and Ene, P.C. (2006). Etiology and prevalence of urinary tract infection in primary school children in Port Harcourt Metropolis. *International Journal of Natural and Applied Sciences*, 2: 362-365.

Mattley, Y.D. and Garcia-Rubio, L.H. (2001). Multi-wavelength spectroscopy for the detection, identification, and quantification cells. *Proceedings SPIE,* 4206: 64-71.

Nakamoto, M., Kunimura, K., Suzuki, J. and Kodera, Y. (2020). Antimicrobial properties of hydrophobic compounds in garlic: Allicin, vinyldithiin, ajoene and diallyl polysulfides (Review). *Experimental and Therapeutic Medicine*, 19: 1550-1553. https://doi.org/10.3892/etm.2019.8388.

Okiki, P.A., Balogun B.D., Osibote, I.A., Asoso, S. and Adelegan, O. (2015). Antibacterial activity of methanolic extract of *Moringa oleifera* Lam. leaf on ESBL producing bacterial isolates from urine of patients with urinary tract infections. *Journal of Biology, Agriculture and Healthcare*, 5(20): 124-132.

Onukak, A.E., Udoette, S.B. and Ekuma, A.E. (2021). Antibiogram of urinary tract pathogens in a tertiary hospital in south-south Nigeria. *Ibom Medical Journal,* 14(2): 191–195.

Rees, L.P., Minney, S.F., Plumer, N.T., Slater, J.H. and Skyme, D.A. (1993). A quantitative assessment of antimicrobial activity of garlic (*Allium sativum*). *World Journal of Microbiology and Biotechnology*, 9: 303–307

Rolf, F.J. (1993). NTSYS-pc Numerical taxonomy and multivariate analysis system. Version 2.0. Exeter Software, Applied Biostatistics Inc. Setauket, New York.

So T.K.A., Abdou, R., Sani, I.S., Toudou, A.K. and Bakasso, Y. (2021). Garlic (*Allium sativum* L.): Overview on its biology and genetic markers available for the analysis of its diversity in West Africa. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 7(3): 1-10.

Stevens, J. (2020). *Pharmacy Times*, 88(9). Available at: https://www. pharmacytimes.com/view/urinary-ract-infections-affect-men-as-well-as-women.

Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner, P., Piot, P. and Heuck, C.C. (2003). *Basic Laboratory Procedures in Clinical Bacteriology* 2nd Edition. World Health Organization Geneva.

Verma, K.S., ulHaq, S., Kachhwaha, S. and Kothari, S.L. (2017). RAPD and ISSR marker assessment of genetic diversity in *Citrullus colocynthis* (L.) Schrad: a unique source of germplasm highly adapted to drought and high-temperature stress. *3 Biotech*, *7*(5): 288.

World Health Organization (2020). Antimicrobial Resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance