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THE EFFECT OF SODIUM HYPOCHLORITE AND ETHANOL AS SEED STERILANTS ON COWPEA INFECTED WITH COWPEA MOTTLE VIRUS

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Abstract

The transmission of viruses through seed can be of considerable ecological significance for virus perpetuation and dissemination. This can also be of economic consequence for the plant as the seeds can be an efficient way by which virus diseases are disseminated in plants. An experiment was conducted to test the efficacy of Sodium hypochlorite and Ethanol at different concentrations as sterilants on cowpea seeds variety 96D-GLO infected with Cowpea Mottle Virus. The cowpea seeds were mechanically inoculated with the virus and surface sterilized at the rate of 5ml inoculum per seed with Sodium hypochlorite (0.5%, 0.75%, 1.0%), Ethanol (75%, 85%, 95%) and distilled water served as control for the experiment. The seeds were then sun dried for 1 hour before sowing into plastic buckets at the rate of 6 seeds per pot. The results indicated that Sodium hypochlorite and Ethanol at different concentrations significantly ($P \geq 0{\cdot}05$) reduced virus disease severity on the plants. This was manifested by increased growth parameters and significantly higher yields compared to the control. The experiment indicated that at the 10th week after planting, Ethanol (95%) had the significantly ($P \geq 0{\cdot}05$) lowest percentage virus disease severity (17.63%), tallest plants (59.13cm), largest number of leaves per plant (72.30) and highest weights for pods (37.17g) and seeds (36.07g). These results indicate prospects for the use of Sodium hypochlorite and Ethanol as seed sterilants in ameliorating viral disease severity on cowpea.

Keywords. Cowpea, disease severity, growth, seed transmission, sterilant, viruses, yield

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is one of the important grain legumes in the world and plays an important role in the livelihood of millions of people in developing countries (El Naim and Jabereldar, 2010). Cowpea has various uses and it is consumed as grain, leaf and forage with high nutritive value and high palatability (Whitebread and Lawrence, 2006). Cowpea is also an important source of food, income and livestock feed. It forms a major component of tropical farming systems because of its ability to improve the fertility status of marginal lands through nitrogen fixation (Timko and Singh, 2008). It has considerable adaptation to high temperatures and drought compared to other crop species, making it suitable for cultivation in semi-arid areas (Tekle, 2014).

Cowpea is a major source of cheap quality protein for both rural and urban dwellers in Africa (Ajeigbe *et al.*, 2012; Dube and Fanadzo, 2013). The leaves and green pods are consumed as vegetable and the dried grain is used in many different food preparations. Protein content of cowpea leaves range from 27 to 43% and protein concentration of the dry grain range from 21% to 33% (Abudulai *et al.*, 2016).

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Cowpea is however susceptible to a number of fungal, bacterial, and viral diseases. Of more than the twenty viruses reported on cowpea from different areas of the world, eight are known to occur in Africa (Lima *et al.,* 2011) and seed transmission is said to account for about one-seventh of the known viruses in one or more of their hosts (Hull, 2002).

Seed transmission plays a pivotal role in the spread and survival of a number of important plant viral diseases. Infected seed is probably the most important source of viruses and sub-viral pathogens in commercial plantings. In fields, the seedlings raised from the randomly dispersed infected seeds serve as initial sources of virus inoculum or foci of infection from which secondary spread occurs within and outside the field by suitable vectors. Besides being a source of inoculum, the seed also helps in perpetuation of the virus over long periods (Chalam and Khetarpal, 2008).

Pathogenic infections can infect seeds internally and destroy the endosperm and the embryo or contaminate the seeds and affect seedling development. Seed borne pathogens primarily cause disease of seeds and have been involved in seed rots during germination and seedling mortality leading to poor crop stand reduction in plant growth and productivity of crops (Akranuchat *et al.,* 2007).Apart from this, infected seeds act as a vehicle in carrying pathogens to uninfected areas within a country and from one country to the other (Waller, 2002; Albrechtsen, 2006). Seed sterilization is therefore an important process that provides insurance against seed-borne as well as soil-borne plant pathogens and insects (Gwary *et al.,* 2007).

In the case of plant viral diseases, different chemical and physical treatments have been reported to eradicate or significantly reduce the incidence of a number of viruses without affecting seed quality. In an experiment by Rast and Stijger (1987), the sterilization of pepper seed infected with Capsicum Mosaic Virus (CaMV) by immersion in 100 g/1 $Na_3 P0_4$ solution was compared with dry heat treatment at 76°C. The virus content of the seed varied with the CaMV strains used to infect the pepper cultivars and the time of harvest of seeds from infected plants.Some other reports indicate that although some seed companies currently utilize pre-treatments seeds, the details of these seed treatment protocols are however proprietary (Córdoba-Sellés *et al.,* 2007).

The objectives of the study were to test the efficacy of Sodium hypochlorite and Ethanol as seed sterilants on cowpea variety 96D-GlO infected with Cowpea Mottle Virus and assess the effect on growth and yield of the crop. It will also quantify the concentration of the sterilant that most significantly reduced severity of the viral disease.

MATERIALS AND METHODS

Source of seed variety and virus inoculum

The potted experiment was carried out in the screenhouse of the Crop Protection Department, Faculty of Agriculture, University of Ilorin – Nigeria. Cowpea variety 96D-GLOand Cowpea Mottle Virus (CPMV) inoculum wereobtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Soil sterilization procedure

The soil used was steam sterilization to a temperature of 80°C for 30minutes, followed by an 8-min resting period resulted in 100% kill of all weeds and soil pathogens (van Loenen *et al.,* 2003). The soil was then potted into plastic perforated buckets of 10 litre capacity prior to the sowing of the treated inoculated seeds.

Virus inoculation and seed Sterilization

The cowpea seeds were firstly mechanical inoculated with Cowpea Mottle virus (CPMV) by slightly dusting the seeds with carborundum to act as slight abrasive agent. This was followed bygently rubbing the seeds with the viral inoculum using cotton wool at the rate of 5ml inoculum per seed. The seeds were thereafter sun dried for 1 hour before sowing into plastic buckets at the rate of 6 seeds per pot (Olaitan *et al*., 2009).

The sterilants were at the following three concentrations: Sodium hypochlorite (NaClO) 0.5%, 0.75%, 1.0%; Ethanol (C_2H_6O) 75%, 85%, 95% and Distilled water served as control for the experiment.

Each treatment was replicated five times and the pots werearranged on raised platforms while irrigation was donetwice in three days until the emergence of flowering.

Data collection and Statistical analysis

Data collected from the 3rd to 10th week after planting (WAP)were: plant height (cm), number of leaves per plant, number of leaves showing characteristic virus disease symptoms (such as mild to severe chlorotic mottling, mosaic patterns and distortion), the percentage virus disease severity measured by the number of diseased leaves relative to the total number of leaves on any given plant and weight of pods and seeds per plant at 80 days after planting.

All collected data were subjected to analysis of variance (ANOVA) using the Statistical Package for the Social Sciences SPSS version 21and the means if significant, separated using the New Duncan Multiple Range Test at 5% level of probability.

RESULTS

Effect on virus severity

Table 1 shows the effects of the treatments on the severity of virus on cowpea seedlings at different times after planting. The results showed that there were significant ($P \leq 0{\cdot}05$) differences between treatments in their efficacy to limit virus severity. Disease initiation for all treatments started at the 4th WAP and the significantly ($P \leq 0{\cdot}05$) lowest percentage virus disease severity were in plants sterilized with Ethanol. Further scrutiny of the values indicated that at 4th,5th, 6th, 7th, 8th, 9thand 10thWAP, sterilization of cowpea seeds with 95% ethanol provided the best protection with the significantly ($P \leq 0{\cdot}05$) lowest disease severity values of 1.4%, 3.7%, 6.73%, 15.43%, 16.83% and 17.63% respectively. The next most effective disinfectant in ameliorating virus disease severity after Ethanol was Sodium hypochlorite. It was also observed (Table 1), that NaClO was significantly ($P \leq 0{\cdot}05$) more effective than distilled water especially at a concentration of 1%. At the 10th WAP, sterilizing cowpea with NaClO at 1% concentration resulted insignificantly ($P \leq 0{\cdot}05$) lower virus disease severity (26.17%) compared with distilled water (39.77%).

Table 1: Effect of treatment on disease severity

	Weeks After Planting (WAP)						
Treatment	**4wk**	**5wk**	**6wk**	**7wk**	**8wk**	**9wk**	**10wk**
NaClO 0.5%	9.80^{a}	13.53^{b}	16.03^{b}	24.37^{b}	29.20^{b}	30.73^{b}	32.60^{b}
NaClO 0.75%	6.23^{c}	11.67^{c}	15.27^{b}	22.33^{c}	26.57^{c}	29.80^{b}	31.40^{c}
NaClO 1%	3.70^{d}	9.27^{d}	12.57^{c}	18.13^{d}	23.27^{d}	24.73^{c}	26.17^{d}
Ethanol 75%	3.40^{d}	6.57^{e}	11.67^{c}	18.57^{d}	22.90^{d}	24.27^{c}	24.93^{e}
Ethanol 85%	2.77^{e}	5.17^{f}	8.90^{d}	14.20^{e}	18.37^{e}	20.40^{d}	21.53^{f}
Ethanol 95%	1.40^{f}	3.70^{g}	6.73^{e}	11.47^{f}	15.43^{f}	16.83^{f}	17.63^{g}
Control	8.43^{b}	15.50^{a}	19.80^{a}	26.27^{a}	32.37^{a}	37.37^{a}	39.77^{a}
S.E.M	0.649	0.922	0.925	1.118	1.233	1.433	1.545

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at $P \geq 0.05$. NS signifies not significant.

Effect on plant height

The effect of treatments on plant height from 4th to 10th WAP is shown in Table 2. The effect ofthe treatments on plant height was apparent from the 5thto 10th WAP. The tallest plants at the 5th WAP were in the treatment with 95% Ethanol (30.27cm), 85% Ethanol (30.03cm), and 1% NaOCl (29.30cm) and these values were not significantly different ($P \geq 0.05$) from Ethanol 75% (27.77cm), NaOCl 0.5% (27.63cm) and NaOCl 0.75% (27.33). The significantly shortest plants were the control (25.73cm). The same trend was observed from the 6th to 10th WAP, whereby the significantly tallest plants were in the treatments with Ethanol 95% (39.57 – 59.13cm) and shortest plants in the

control with values ranging from 29.47 to 40.73cm.

Table 2: Effect of treatment on plant height (cm)

	Weeks after Planting							
Treatment	**3wk**	**4wk**	**5wk**	**6wk**	**7wk**	**8wk**	**9wk**	**10wk**
NaClO 0.5%	22.43	25.37	27.63^{ab}	30.13^{e}	33.63^{e}	39.80^{e}	42.37^{d}	43.90^{e}
NaClO 0.75%	22.30	24.13	27.33^{ab}	32.23^{d}	35.53^{d}	41.90^{d}	44.07^{d}	45.93^{d}
NaClO 1%	21.80	24.37	29.30^{a}	35.97^{bc}	42.37^{c}	48.40^{c}	50.40^{c}	52.97^{c}
Ethanol 75%	21.17	23.83	27.77^{ab}	35.23^{c}	41.67^{c}	47.13^{c}	48.80^{c}	51.43^{c}
Ethanol 85%	22.57	24.50	30.03^{a}	37.33^{b}	44.67^{b}	51.53^{b}	53.43^{b}	55.17^{b}
Ethanol 95%	20.30	22.43	30.27^{a}	39.57^{a}	48.07^{a}	55.57^{a}	57.57^{a}	59.13^{a}
Control	21.87	23.63	25.73^{b}	29.47^{e}	32.53^{e}	36.23^{f}	38.87^{e}	40.73^{e}
S.E.M	0.368	0.368	0.453	0.808	1.237	1.427	1.371	1.378

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at P≥0.05.

Effect on number of leaves per plant

The results of the effect of the treatments on mean number of leaves per plant (Table 3) revealed that there were significant differences among the treatment means from 6th to 10th WAP. The seeds treated with Ethanol at 95% produced the significantly highest average number of leaves per plant (58.17 to 72.30) for the duration of the experiment.

Table 3: Effect of treatment on number of leaves per plant

	Weeks after Planting (WAP)							
Treatment	**3wk**	**4wk**	**5wk**	**6wk**	**7wk**	**8wk**	**9wk**	**10wk**
NaClO 0.5%	21.70	32.70	43.87	48.63^{c}	54.03^{c}	59.33^{c}	62.30^{c}	63.33^{c}
NaClO 0.75%	20.73	31.80	44.03	49.27^{c}	54.17^{c}	59.30^{c}	62.27^{c}	63.63^{c}
NaClO 1%	21.27	30.20	44.77	50.10^{c}	56.50^{bc}	62.70^{b}	65.70^{b}	66.87^{b}
Ethanol 75%	20.43	31.17	44.80	49.70^{c}	56.10^{bc}	62.63^{b}	65.50^{b}	66.53^{c}
Ethanol 85%	21.13	31.87	44.30	52.80^{b}	59.27^{b}	61.67^{b}	64.93^{b}	66.07^{b}
Ethanol 95%	21.03	32.60	44.77	58.17^{a}	65.40^{a}	67.30^{a}	71.47^{a}	72.30^{a}
Control	22.60 NS	31.57 NS	44.17 NS	49.17^{c}	55.03^{c}	61.30^{b}	64.57^{b}	67.30^{b}

NS = not significant

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at P ≥0.05.

Effect on yield parameters

The effect of the treatments on yield parameters as shown in Table 4 indicated that the significantly highest number of pods (37.17g) and seed weights (36.07g) were obtained in the treatment with 95% Ethanol compared to an average number of 8.63 pods per plant and 7.7g seed weight obtained in the control treatment.

Table 4: Effect of treatment on yield

Treatment	Yield Parameters (g) Wt. of pods/plant	Wt. of seeds/plant
NaClO 0.5%	12.77[d]	11.47[d]
NaClO 0.75%	14.73[d]	13.37[d]
NaClO 1%	20.47[c]	19.00[c]
Ethanol 75%	20.40[c]	18.97[c]
Ethanol 85%	25.30[b]	23.47[b]
Ethanol 95%	37.17[a]	36.07[a]
Control	8.63[e]	7.70[e]
S.E.M	1.965	1.953

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at P $\geq$0.05.

Discussion

The present study affirms the efficacy of Ethanol and Sodium hypochlorite in ameliorating virus disease severity in cowpea. The elucidation of the exact mechanism of action of disinfectants against plant viruses could be difficult because viruses are template nucleic acid molecules embedded in lipo-protein bilayer. It can be assumed therefore that the ability of the sterilants to ameliorate virus severity in cowpea variety 96D-GLO was due to the concentration of hydrogen (H^+) and hydroxyl (OH^-) ions in both Ethanol and Sodium hypochlorite. The H^+ ions most probably destroyed the amino – acid bond in the nucleic acids of the virus thereby modifying the cytoplasmic pH, thus leading to the precipitation and dissolution of the virus protein.

The OH^- ions could also have caused the saponification of some lipids in the enveloping membrane, thus leading to the destruction of the superficial structure and subsequent hydrolysis of the nucleotides of the virus genome. This view is also shared by Dauphin and Darbord (1988) on the actions of some sterilants against plant virus viability. Kuyyakanond and Quesnel (1992) had earlier postulated that the presence of lipids in a virus is uniformly associated with a high degree of susceptibility to disinfectants and the absence of lipid and small size are associated with resistance to lipophilic chemical agents and sterilants. The report is consistent with Hu *et al.* (1994) which also found that undiluted skim milk and commercial bleach used as seed sterilantsat 10% or 20% concentration inactivated Cymbidium Mosaic Virus (CyMV) and Odontoglossum Ringspot Virus (ORSV) on a local lesion host.

The observation of action of the sterilants on height and number of leaves of the plants revealed that seeds treated with either Ethanol or Sodium hypochlorite had significantly taller plants and produced more leaves. This probably suggests that both sterilants improved plant growth by increasing germination time. This finding is in agreement with submissions by Ho *et al.* (1995) and Pernezny *et al.* 2002. They also opined that Sodium hypochlorite, Phenolic and Formaldehyde compounds accelerated germination and improved the rate of plant growth in all the cases considered. Similar results were also reported by Sen *et al.* (2013).

In most instances disease is logically related to yield and disease severity is negatively correlated with yield. This implies that the growing and production status of plants is affected by their level of disease susceptibility. This study verified that cowpea seed sterilization with Ethanol and Sodium hypochlorite cause reduction in disease severity and eventual higher plant yields. The effectiveness of the

disinfectants to ameliorate disease severity is a considerable aspect of yield verification. This can be deduced as the basis for the higher pod and seed weights obtained in the study. This observation is in agreement with those made by Jan *et al.* (2013), who in a study reported that surface sterilization produced higher yields of field grown strawberry explants intended for in vitro culture.

Conclusion

The study has presented at first glance, the effect of Sodium hypochlorite and Ethanol at different concentrations as seed sterilants on cowpea seeds mechanically infected with Cowpea Mottle Virus. The study established that the sterilants were the main reason for the reduction in viral disease on the cowpea plants. This reduction subsequently resulted to improvements in the growth and yield capacity of the crop. The effect was however more assertive in surface sterilization with Ethanol (95%). The outcome of this study shows greater prospect in the application of Ethanol (95%) as seed sterilant to control virus diseases in cowpea.

Conflict of interest

Author declares no conflict of interest.

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Anti-inflammatory Activity of the Fractions from the Methanol Extract of *Carpolobia lutea* G. Don (Polygalaceae) Fruit and Leaf

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Abstract

The use of nonsteroidal anti-inflammatory drugs (NSAIDS) is not applicable to all patients and cases, most especially in chronic inflammatory condition, because of the adverse effects which include an increased risk of ulcerative diseases, kidney and heart diseases**.** Therefore, this study is to evaluate the fractions from the methanol extract of the fruit and leaf of *Carpolobia lutea* for anti-inflammatory effect. The anti-inflammatory activities were evaluated using two models: inhibition of albumin denaturation and membrane stabilization test. The results showed that the different solvent fractions of *Carpolobia lutea* leaf and fruit possessed varying anti-inflammatory properties, at inhibiting the heat induced albumin denaturation and stabilizing the Red Blood Cells membrane at 200 and 1000 µg/ml respectively. The leaf was more active than the fruit part of the plant at inhibiting inflammation for both experimental models. Maximum inhibition (64.3 %) was observed for the n-hexane fraction of the leaf at 1000 µg/ml for the heat induced albumin denaturation while the chloroform and ethyl acetate fractions of the leaf and fruit were not active at both concentrations. The maximum inhibition was recorded for the chloroform fraction (65.54 %) of the plant leaf at the concentration of 200µg/ml while the n-hexane fraction was not active for the membrane stabilization test. These findings justify the ethnomedical use of this plant as an anti-inflammatory agent.

Keywords: *Carpolobia lutea*, anti-inflammatory, albumin denaturation, fractions, membrane stabilization

INTRODUCTION

Plants, which are sources of natural products, have provided promise of cure for various ailments, as they have produced the lead compounds for the synthesis/biosynthesis of drugs and which is an important source of new therapeutic agents (Andreo *et al*., 2006). Inflammatory diseases including rheumatism and arthritis continue to be well established medical problem and a major cause of morbidity worldwide. Since the finding of aspirin from Willow's bark, more than 100 years ago, many steroidal, as well as non-steroidal anti-inflammatory drugs have been introduced such as ibuprofen, naproxen and diclofenac e.t.c However, the prolonged use of most of these prescription drugs reportedly causes renal diseases, gastrointestinal irritation and other adverse effects (Bertolini *et al.*, 2001). Thus, global interest has been aroused to discover potential anti-inflammatory molecules from plants which are traditionally used for aches, fever and rheumatic pain (Basu and Hazra, 2006).

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Carpolobia lutea G. Don (Polygalaceae) is known as cattle stick in English, Ikpafum (Ibibio), Agba or Angalagala (Igbo) and Egbo Oshunshun (Yoruba) in Nigeria (Ajiwhen and Bisong, 2013). The shrub, *Carpolobia lutea*, (*C. lutea*) is regarded traditionally as remedy for a lot of ailments such as sterility, dermal and venereal disease, worm infestation, diarrhoea, malaria, rheumatism, snake bite, insanity and as aphrodisiac (sexual invigorator) among other uses (Yakubu and Akanji, 2014). Ethnomedical survey of anti-inflammatory plants sold in Lagos Nigeria herb markets reveals that the stem bark, leaves and roots of the plant are used for this purpose (Sofidiya *et al.,* 2014). *C. lutea* root decoction is widely used by traditional herbal practitioners as malarial remedy (Nwafor and Bassey, 2007; Ajibesin *et al.,* 2008), anti-inflammatory/anti-arthritic (Iwu and Anyawu, 1982), anti-inflammatory and antipyretic of the leaf Ndiwu and Nwafor, 2012, analgesic activity (Jackson *et al.,* 2011), anthelmintic and antisterility agent (Mitaine *et al.,* 2002)

Nonsteroidal anti-inflammatory drugs (NSAIDS) are often not suitable in all patients and cases, particularly in chronic inflammatory condition, because of their major adverse effects such as ulcerative and renal diseases. Hence, there is need for continuing search for alternative remedies**.** It has also been observed that not much work has been done on the anti-inflammatory efficacy of the fruit and leaf fractions of the plant used in this study. Hence, the aim of this study is to evaluate the fractions from the methanol extract of fruit and leaf of *Carpolobia lutea* for anti-inflammatory activity.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves and fruits of *Carpolobia lutea* used in the study were collected from Surulere estate, Iyesi- Otta, Ado- Odo Local Government Area of Ogun State, Nigeria. The plant was identified at the Department of Pharmacognosy, University of Lagos, Nigeria with voucher specimen number PCGH-35. The test plant parts were washed, cut into small pieces and air dried under the shade for seven weeks until completely dried. The dried plant materials were ground into powder and stored in air-tight glass bottles at room temperature prior to experiments.

Extraction procedure

The powdered *Carpolobia lutea* leaf (250 g) were then subjected to cold maceration in a total of 1,500 ml of solvent system containing methanol (Analar grade) 750 ml and distilled water 750 ml over 72 hrs. The crude extract was filtered first through cotton wool, then through Whatman's filter paper of 125 mm. The filtrate was then concentrated using rotary evaporator at 35°C and further dried in the oven at 35°C. This same procedure was repeated for 330g of the powdered fruit. The dry extracts were weighed, stored in a sample bottle and preserved in the freezer before fractionation.

Extraction and Fractionation

The leaf and fruit extracts, 40g each were dissolved in 500 ml of distilled water and placed in a separating funnel to be fractionated with 3 × 500 ml of each solvent in order of polarity, starting with the least polar, n-Hexane, followed by chloroform and ethyl acetate. The fractions were dried in the oven at 35°C. The dried fractions were weighed and stored in a glass sample bottle for further experiment.

In vitro Anti-inflammatory activity

Inhibition of Albumin Denaturation:

Methods of Mizushima and Kobayashi, 1968 and Sakat *et al*., 2010 were ensured with minor modifications. The reaction mixture consists of the test extracts (at 200 and 1000 µg/ml) and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted using a small amount of HCl at 37ºC. The sample extracts were incubated at 37°C for 20 mins and then heated to 51ºC for 20 minutes. After cooling the samples, the turbidity was measured spectrophotometrically at 660nm with a double beam UV-Visible Spectrometer (PG Instruments Ltd, T80 +, S/N 151885-01-0094).The experiment was carried out in triplicate. Aspirin was used as a standard drug. Percentage inhibition of protein denaturation was calculated using the Eq 1:

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \text{-----Eq 1}$$

Where $\text{Abs}_{\text{control}}$ is the absorbance without sample, $\text{Abs}_{\text{sample}}$ is the absorbance of sample extract/ standard.

Membrane Stabilization Test:

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected from a volunteer and transferred to the laboratory centrifuge and was spin at 3000 rpm for 10 min and were washed three times with equal volume of normal saline and reconstituted as 10% v/v suspension with normal saline (Sakat *et al.*,2010; Sadique *et al.*, 1989). The study was conducted in accordance with the nationally accepted guidelines for release of blood for non-clinical purposes and approval was given by the College of Medicine, University of Lagos, Nigeria Health Research Ethics Committee with approval number CMUL/HREC/07/17/209.

Heat Induced Haemolytic: The reaction mixture (2ml) consist of 1ml of test sample solution (200 and 1000 µg/ml) and 1ml of 10% RBCs suspension. Instead of test sample, only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56^{o}C for 30 mins. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates for all the test samples. Percentage membrane stabilization activity was calculated by the formula of E.q 1. (Saka *et al*., 2010; Shinde *et al.* 1999).

RESULTS

The weight of the *C. lutea* leaf extract was 63.55 g and fruit extract 46.17 g which translate to 19.26% and 16.13% yield respectively.

Inhibition of albumin denaturation

Maximum inhibition (64.3 ± 0.01) was observed from the n-hexane fraction of the leaf at 1000 µg/ml concentration followed by fruit (42.9 ± 0.0005) at 200 µg/ml concentration (table 1). Chloroform and ethyl acetate fractions of the *C. lutea* leaf and fruit were not active at both concentrations. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 61.2 % at the concentration of 1000 µg/ml (table 1).

Membrane stabilization test

The fractions inhibited the heat induced haemolysis of RBCs to varying degree (Table 2). The maximum inhibition was recorded from chloroform fraction (65.54 %) of the plant leaf at the concentration of 200 µg/ml followed by 64.7 % at 1000 µg/ml and ethyl acetate fraction (61.73 %) of the fruit at 1000µg/ml, while the n-hexane fraction of the leaf was not active. Aspirin, a standard anti-inflammation drug showed inhibition of 86.92 % at the concentration of 1000 µg/ml (table 2).

Table 1: **Percentage inhibition of different solvent fractions of the *C. lutea* leaf and fruit on albumin denaturation inhibition**

Test fractions	Leaf		Fruit	
	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml
n-Hexane	na	64.3 ± 0.01	42.9 ± 0.0005	na
Chloroform	na	na	Na	na
Ethyl acetate	na	na	Na	na
Aspirin acid	60.7± 0.0005	61.2± 0.0005	60.7± 0.0005	61.2± 0.0005

Note: na = not active

Table 2: Percentage Inhibition **of different solvent fractions of the *C. lutea* leaf and fruit on membrane stabilization inhibition**

Test fractions	Leaf		Fruit	
	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml
n-Hexane	na	na	14.06±0.0012	na
Chloroform	65.54±0.0007	64.7±0.0005	11.63±0.0006	na
Ethyl acetate	48.63±0.0089	na	32.77±0.0053	61.73±0.0024
Aspirin	85.96±0.002	86.92±0.002	85.96±0.002	86.92±0.002

Note: na = not active

DISCUSSION

In this study anti-inflammatory activity of fractions from *C. lutea* leaf and fruit extracts were evaluated using two different experimental models. Denaturation of proteins is a well-documented cause of inflammation (Mizushima and Kobayashi, 1968). The inflammatory drug (Aspirin) has been reported to show dose dependent ability to thermally induce protein denaturation (Mizushima and Kobayashi, 1968). Similar results were observed from this study with percentage inhibition of 60.7 % and 61.2 % at concentration of 200 and 1000 µg/ml respectively (table 1). The n-hexane fraction of *C. lutea* leaf was the most effective with a maximum inhibition of 64.3 % at concentration of 1000 µg/ml followed by the fruit (42.9 %) at concentration of 200µg/ml; this shows that the leaf was more active at a higher concentration than the fruit part of the plant at inhibiting inflammation for this type of experimental model (table 1). These fractions may likely inhibit the release of lysosomal content of neutrophils at the site of inflammation. These neutrophils lysosomal component include bactericidal enzymes and proteinases, which upon extracellular release cause advance tissue inflammation and injury (Chou, 1997). .

The results of the stabilization of RBCs membrane revealed that the maximum inhibition of the heat induced haemolysis was recorded from chloroform fraction (65.54 %) of the leaf at the concentration of 200 µg/ml followed by (64.7 %) at 1000 µg/ml (table 2). This result is verification for membrane stabilization as a mechanism of anti-inflammatory effect. This also shows that the leaf part was further active than the fruit part of the plant at inhibiting inflammation for this type of experimental model. This effect may perhaps inhibit the release of lysosomal content of neutrophils at the site of inflammation. However, the definite mechanism of this membrane stabilization is yet to be elucidated; it is possible that the chloroform fraction of the leaf produced this effect of surface area to volume ratio with the cells, which likely brought about an increase in size of membrane or the shrinkage of cells and an interaction with membrane proteins (Shinde *et al*., 1999).

Furthermore, the study of Ndiwu and Nwafor *et al*., 2012, also supported the anti-inflammatory activity of fractions from *C. lutea* leaf. The n-hexane, chloroform, ethylacetate and ethanol, fractions possessed inhibitory effects on the acute phase inflammation using the following experimental models: formalin test, egg albumin test, capsaicin test, xylene-induced ear edema test and carragenin test in rats. The ethanol fraction revealed the maximum inhibitory effect of 52.37 % followed by chloroform with 52.29 % at 770 mg/kg dose for the xylene-induced ear edema test.

CONCLUSION

In the present investigation, it was revealed that certain fractions of *Carpolobia lutea* leaf and fruit can be a potential source of anti-inflammatory agents by inhibiting the heat induced albumin denaturation and stabilizing the Red Blood Cells membrane at 200 and 1000 µg/ml respectively. The leaf part was more active than the fruit part of the plant at inhibiting inflammation for both experimental models.

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Declaration of Interest statement

The authors declare no conflict of interest concerning this research studies.

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Effect of *Dialium guineense* Fruit Pulp Meal-based Diet on Haematological parameters, Lipid profile and Organ/body weight ratio of Rats

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Abstract

Haematological, lipid profile and organ/body weight ratio are some of the biochemical parameters used in assessing the toxicity of a food/drug. The study aimed at determining these parameters in albino rats fed the fruit pulp of *Dialium guineense* at 5% - 40 % inclusion levels. 36 rats of both sexes were grouped into 6 (A – F). A served as the control while B – F where maintained on *Dialium guineense* fruit pulp meal-based diet at 5% - 40% supplementation respectively for five weeks. Haematological and lipid Profiles were determined using standard methods while Organ/body weight ratio was expressed in percentage. The study revealed no alteration in almost all the haematological parameters of the test groups when compared with the control except for lymphocytes in which a significant increase ($p < 0.05$) (50.72 ± 1.73%) was observed at 40% supplementation while for neutrophils, significant increase and decrease ($p < 0.05$) was observed in the group fed on 30%; 40% diet (increase)(47.68 ± 2.13%, 48.11 ± 2.20%) and 5% diet (decrease) (41.68 ± 0.99%) respectively. For the lipid profile, a significant increase was observed at all inclusion levels in the total cholesterol and high density lipoprotein concentration while there was a significant decrease ($p < 0.05$) in the low density lipoprotein concentration, triglyceride and atherogenic index at all inclusion levels of the fruit pulp. For the Organ body weight ratio, no significant difference ($p > 0.05$) was observed in all the groups. The results suggest that the diet might not exhibit structural toxicity on selected organs but portray indications of minute functional toxicity which are still within the tolerable range.

Keywords: Haematological parameters, *Dialium guineense*, Lipid profile, Atherogenic index.

Introduction

Reference ranges of physiological parameters can be useful for the evaluation of the state of health in specimens of the species as well as diagnostics and prevention of diseases (Tomenendalova, 2014). Haematology refers to the study of the numbers and morphology of the cellular elements of the blood and the use of these results in the diagnosis and monitoring of disease with the respective damage to blood (Merck, 2012, Onyeyili *et al*., 1992, Togun *et al*., 2007). Haematological parameters are used to assess the status of the blood and are related to the blood and blood-forming organs (Stenesh, 1975). Their elevated or decreased level has various clinical implications. They include Red blood Cell Counts, Haemoglobin, Haematocrit, Mean Corpuscular haemoglobin, Mean Corpuscular Haemoglobin Concentration, White blood Cell Count, Platelet Counts e.t.c. (NseAbasi, 2014).

Blood is a vital substance made up of red (erythrocytes) and white blood cells (leukocytes) suspended in a liquid called blood plasma. The blood of mammals contains many proteins which are involved in blood clotting. Blood circulates around the body in arteries and veins; and acts as a transport system for many substances including oxygen, amino acids (proteins), lipids (fats), sugar, glucose (carbohydrate), hormones and waste products (ammonia and carbon dioxide). Blood act as a pathological reflector of the status of the exposed animals to toxicants and other conditions (NseAbasi, 2014).

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Blood is a vital substance made up of red (erythrocytes) and white blood cells (leukocytes) suspended in a liquid called blood plasma. The blood of mammals contains many proteins which are involved in blood clotting. Blood circulates around the body in arteries and veins; and acts as a transport system for many substances including oxygen, amino acids (proteins), lipids (fats), sugar, glucose (carbohydrate), hormones and waste products (ammonia and carbon dioxide). Blood act as a pathological reflector of the status of the exposed animals to toxicants and other conditions (NseAbasi, 2014).

Serum is the fluid portion of the blood obtained when the blood is allowed to clot in the absence of an anticoagulant (Yakubu, 2006). It is free of clotting proteins but contains the clotting metabolites that result from the clotting process. It is a cleaner sample typically free of cells and platelets because they are trapped in the fibrin meshwork of the clot. The serum is responsible for the homeostasis process within the cell (Ganong, 2001). It contains proteins like albumin and globulin (Murray *et al*., 2000), and immense number of ions, inorganic and organic molecules that are in transit to other parts of the body and thus aid transport of other substances (Ganong, 2001). It also contains water, proteins, glucose, lipids, amino acids, enzymes, hormones, antigens and urea (Ganong, 2001). Lipids are fatty substances that are normally insoluble in an aqueous environment but are solubilised and transported in the plasma as water-soluble macromolecular complexes known as lipoproteins (Styrer, 1995). Lipids found in the serum that are of diagnostic importance include cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and phospholipids (Nelson and Cox, 2000).

An important requirement in toxicological experiments is the ability to assess the effects of xenobiotics on specific organs. For many organs, this is done through macroscopic examination of the organs, measuring organ weight, and histopathological examination of the tissue (Bailey *et al*., 2004). Organ weight can be the most sensitive indicator of an effect of an experimental compound, as significant differences in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes (Bailay *et al.*, 2004). The organ-body weight ratio is the ratio of the organ to the body weight of an animal (Green, 1980) and it is used in predicting clinical conditions. It is a 'marker' of cell constriction and inflammation (Moore and Dalley, 1999) and is normally investigated to determine whether the size of the organ has changed in relation to the weight of the whole animal (Adebayo *et al.*, 2003) and this could reflect the state of the organ and consequently its function.

Medicinal plants were considered by several researchers to form an important component of the natural wealth of the country (Egharevba and Ikhatua, 2008). In fact, most plants produce a diverse range of bioactive molecules making them rich sources of medicinal compounds which have continued to play a dominant role in the maintenance of human health since ancient times. These bioactive molecules termed phytochemicals are non-nutritive plant chemicals that possess the curative properties ((Egharevba and Ikhatua, 2008).

Dialium guineense (Wild) belongs to the family of *Fabaceae.* It is known as Velvet or Black tamarind (English), Awin (Yoruba), Icheku (Igbo), Tsamiyar kurmi (Hausa) (Aiyeloja and Bello, 2006). It is a woody plant that grows up to 15 m high in the rain forest region of West Africa (Okegbile and Taiwo, 1990). It has densely leafy crown, smooth greyish bark. Leaves are hairy and the flowers are usually whitish while the fruits are less circular and flattened. The small black velvet fruits are very conspicuous and distinctive. Fruits usually abundant, more or less circular and flattened, but sometimes almost globose, up to 2.5 cm in diameter, densely velvety, black; each fruit with a stalk about 6 mm long with a little collar near the apex, with a brittle shell enclosing 1 seed (or exceptionally 2), embedded in a dry, brownish, sweetly acidic, edible pulp (Aiyeloja and Bello, 2006). The fruit pulp which is yellowish-red in colour has a sweet-sour, astringent flavour similar to baobab but sweeter than it and it is eaten raw by man and animal.

The decoction of the bark of *Dialium guineense* is prescribed for stomach-ache and mouth wash (Gills, 1992). The leaves of *Dialium guineense* has also been reported for the treatment of cough and fever (Gills, 1992).. Adjanohoun *et al* (1989) reported the anti-diarrhoeal activity of the aqueous root extract of *Dialium guineense*. Odukoya *et al* (1996) also reported the anti-microbial activity of the ethanolic extract of the leaves of *Dialium guineense* plant. The fruit pulp of the plant is claimed locally to have anti-ulcerogenic potential and this has been scientifically authenticated by Oyegoke and Oladiji (2014) with the determination of the secondary metabolite constituents of the aqueous extract, proximate constituent of the fruit pulp and the formulated feed.

However, after authenticating the efficacious use of the fruit pulp of this plant as an anti-ulcer agent, we realize that there is dearth of information on its toxicological implications in open scientific literature; therefore, there is need to provide information to fill this lacuna. Therefore, the overall objective is to evaluate the safety of *Dialium guineense* fruit pulp in rats as it relates to haematological parameters, lipid profile and Organ body weight.

Materials and Methods

Plant Material and Authentication

The fruit pulp of *Dialium guineense* purchased from Dawanu Market in Kano City, Nigeria was authenticated in the Department of Plant Biology, University of Ilorin,

Ilorin, Nigeria. A voucher specimen (UIH1064) was deposited in the Herbarium of the Department.

Laboratory Animals

Thirty six albino rats (*Rattus novergicus*) of both sexes (167.09 ± 6.78g, 5-7weeks old) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were kept in well-ventilated house conditions with free access to rat pellets (Premier Feed Mills Company Limited, Ibadan) and tap water before the commencement of the experiment.

Feed Ingredient

Yellow maize (*Zea mays*) seed, cellulose (corn corb) and soybean were obtained from 'Oja Oba' market in Ilorin, Nigeria. Soybean oil was a product of Sunola Refined Soybeans, Kewalram Nigeria Limited, Nigeria. Vitamin/ Mineral mix, Lysine and D-Methionine were products of Rofat Feed Nigeria Limited, Ilorin, Nigeria.

Assay kits, Chemicals and Reagents

Assay kits for Cholesterol, Triglyceride, High and Low Density lipoprotein, chemicals and reagents used for this research were of analytical grade and were obtained from Randox laboratories Co-Atrim, United Kingdom.

Preparation of *Dialium guineense* fruit pulp powder

Fresh fruit pulps of *Dialium guineense* were manually removed from the seed after expunging it from the seed coat. They were then oven-dried at 40^{o}C and pulverized in an electric blender (Super Master Electrical Appliance Industries Co., Kyoto, Japan) and then stored for later use.

Composition of Diets

The feed ingredients were weighed out, thoroughly mixed manually until it was homogenous. The feed were then made into pellets and packed in polythene bags, labelled and stored in the refrigerator. The composition of the diet is shown in Table 1.

Table 1: Composition of the Diet

	Dialium guineense fruit pulp meal-based diet (%)					
Ingredient	Control (Basal Diet)	5	10	20	30	40
Corn starch	506	480.70	455.40	404.80	354.20	303.60
D. guineense fruit pulp	-	25.30	50.60	101.20	151.80	202.40
Cellulose	40	40	40	40	40	40
Sucrose	100	100	100	100	100	100
Soybean	250	250	250	250	250	250
Soybean Oil	50	50	50	50	50	50
Vitamin/Mineral	50	50	50	50	50	50
D-Methionine	4	4	4	4	4	4

***** Vitamin/Mineral mix: Vitamin A 4,000,000 i.u; Vitamin D_3, 800,000 i.u.; Tocopherols, 400 i.u; Vitamin K_3 800mg , Folacin, 200mg; Thiamine, 600mg; Riboflavin 1,800mg; Niacin, 6000mg; Calcium pathothenate, 4 mg; Biotin, 8 mg; Manganese, 30,000mg, Zinc, 20,000mg; 8,000mg; Choline chloride 80,000mg; Copper, 2,000mg; Iodine, 480mg; Cobalt, 80 mg; Selenium, 40mg; BHT, 2,500mg. Anticaking agent, 6000mg. Unit of diet composed – g/ kg.

Animal Grouping

Albino rats (36) were acclimatized for one week and were kept in well-ventilated animal house conditions (temperature: 22 ± 3^{o}C; photoperiod: 12hrs light and 12hrs dark; humidity: 40 – 45%) with free access to rat pellets (Premier Feed Mills Company Limited, Ibadan, Nigeria) and tap water before the commencement of the experiment. After a week of acclimatization, they were weighed {intial weight (g)} and assigned into six groups of six rats each (Table 2):

Table 2: Animal grouping based on Feed Composition

Group	Feed Composition
A	**Basal (Non-Supplemented) diet**
	Percentage Inclusion of Dialium guineense Fruit Pulp (%)
B	5
C	10
D	20
E	30
F	40

The animals were maintained on their respective diets for a period of five weeks before sacrifice.

Animal Sacrifice, Preparation of Serum and Excision of the Organs of Study

Animals were weighed before sacrifice (Final weight). The procedure described by Yakubu *et al* (2008) was adopted for the animal sacrifice. Under diethyl ether anaesthesia, the veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were cut with a sterile scapel blade and 5ml each of the blood was collected into clean and dry centrifuge tubes and heparinized sample bottles respectively. The blood collected into the centrifuge tube was left undisturbed to clot for 10 minutes at room temperature after which they were thereafter centrifuged at 1282 x *g* for 5 minutes using Uniscope Laboratory centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). The sera were later aspirated with Pasteur pipettes into dry, sample (non-heparinized) bottles and used within 12 hours of preparation for the lipid profile assay. The animals were quickly dissected and the liver and kidney excised, cleaned and weighed.

Determination of haematological parameters

The blood collected into the heparinised sample bottles was used for the determination of haematological parameters. The Automated Haematological Analyser, Sysmex, KX- 21 (Japan) was used to determine the levels of haemoglobin (Hb), red blood cells (RBC), Packed cell volume (PCV), Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC), Mean cell volume (MCV) and White blood cell (WBC). The Stromatolyser counts the red cells, lyses them to release the haemoglobin and estimates this concentration. The machine assumes that all nucleated cells are white cells and therefore counts all as white cells into their different forms i.e. lymphocytes and neutrophils, but will not differentiate between eosinophils, monocytes and basophils and as such, they are recorded as mixed. It lyses the white blood cells

based on the size of the nucleus and counts the number of the white cells.

Determination of serum lipid profile

The total cholesterol concentration was determined by colorimetric technique as described by Allain *et al*. (1974). Triglycerol concentrations in the serum were determined as described by Searcy (1969). HDL-Cholesterol was determined by a colorimeteric technique described by Burstein and Mortin (1969). The serum low-density lipoprotein cholesterol was assayed using the polyvinyl sulphate (PVS) reaction as described by Demacker *et al* (1984). The atherogenic index was computed using the method described by Ng *et al* (1997). The computation was done adopting the expression LDL -Cholesterol/ HDL -Cholesterol.

Organ-body weight ratio (Expressed in percentage)

The animals were weighed before sacrifice. The organs of interest were excised and weighed after blotting each in blotting paper to remove blood and water. The weights were noted and the organ-body weight ratio was composed from the expression:

$$\text{Organ-Body Weight Ratio} = \frac{\text{Weight of the organ}}{\text{Weight of the animal}} \times 100$$

2.12 **Statistical analysis**

Data was expressed as the mean ± SEM of six determinations. All results were statistically analyzed using one-way ANOVA and Duncan's Multiple Range Test (DMRT) (Duncan, 1957). Differences between group means were considered significant at $p < 0.05$.

Results

The effect of *Dialium guineense* fruit pulp meal-based diet on the haematological profile of rats is presented in Table 3. There was no significant difference ($p > 0.05$) in the red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of rats fed on *Dialium guineense* fruit pulp meal-based diet at all levels of inclusions (5% -40%) when compared with the rats maintained on basal diet (control) group. Although, there were no significant difference ($p > 0.05$) in the white blood cell (WBC) at all inclusion levels of the fruit pulp meal-based diet, a significant decrease ($p < 0.05$) was observed in the neutrophil count at 5% inclusion level while a significant increase ($p < 0.05$) was observed at 30% and 40% inclusion levels. Likewise, a significant increase ($p < 0.05$) in the lymphocyte count was also noted at 40% inclusion level of the fruit pulp.

Table 3: Effect of *Dialium guineense* fruit pulp meal-based diet on the haematological profile of rats

	Treatment groups					
Parameters	**Control**	**5% *D.g***	**10% *D.g***	**20% *D.g***	**30% *D.g***	**40% *D.g***
RBC (10^6 /uL)	7.07 ± 0.13[a]	6.85 ± 0.34[a]	6.97 ± 0.24[a]	6.86 ± 0.24[a]	6.91 ± 0.65[a]	6.86 ± 0.35[a]
Hb (g/dl)	14.79 ± 0.04[a]	14.55 ± 0.05[a]	14.57 ± 0.04[a]	14.66 ± 0.08[a]	14.77 ± 0.08[a]	14.83 ± 0.13[a]
PCV (%)	39.15 ± 0.26[a]	38.81 ± 0.79[a]	39.16 ± 0.77[a]	39.28 ± 0.52[a]	38.93 ± 1.44[a]	38.75 ± 1.11[a]
MCV (fl)	62.49 ± 0.28[a]	61.47 ± 1.44[a]	62.18 ± 1.60[a]	61.82 ± 1.85[a]	61.88 ± 0.26[a]	61.72 ± 0.21[a]
MCH (pg)	17.10 ± 0.11[a]	17.04 ± 0.27[a]	16.94 ± 0.53[a]	16.98 ± 0.84[a]	16.99 ± 0.59[a]	17.14 ± 0.34[a]
MCHC (g/dl)	28.62 ± 0.46[a]	28.36 ± 0.58[a]	28.28 ± 0.35[a]	29.00 ± 0.12[a]	28.40 ± 0.11[a]	28.96 ± 0.63[a]
WBC (10^3 /L)	22.29 ± 0.89[a]	22.77 ± 0.75[a]	22.37 ± 0.38[a]	22.26 ± 1.56[a]	22.25 ± 0.97[a]	22.81 ± 0.90[a]
Lymphocytes (%)	47.46 ± 1.89[a]	47.09 ± 0.84[a]	46.90 ± 2.89[a]	46.22 ± 1.05[a]	47.02 ± 2.03[a]	50.72 ± 1.73[b]
Eosinophils (%)	0.48 ± 0.02[a]	0.48 ± 0.02[a]	0.46 ± 0.02[a]	0.48 ± 0.01[a]	0.48 ± 0.01[a]	0.47 ± 0.01[a]
Neutrophils (%)	42.90 ± 1.17[a]	41.68 ± 0.99[c]	42.94 ± 2.09[a]	44.26 ± 2.11[a]	47.68 ± 2.13[b]	48.11 ± 2.20[b]

Data are means of six determinations ± SEM. Values with superscripts different from the control down each column for each parameter are significantly different ($p < 0.05$). *D.g – Dialium guineense* fruit pulp meal-based diet. Key :- D.g – *Dialium guineense* fruit pulp meal-based diet. RBC: Red Blood Cell, Hb - Haemoglobin , PCV – Packed Cell Volume , MCV – Mean Cell Volume , MCH – Mean Cell Haemoglobin , MCHC – Mean Cell Haemoglobin Concentration, WBC – White Blood Cell.

Dialium guineense fruit pulp meal-based diet at all the inclusion levels significantly increased ($p < 0.05$) the concentration of total cholesterol (TC) and high density lipoprotein cholesterol (HDL–C) compared with those maintained on basal diet (Table 4). For the total cholesterol concentration, rats fed on 5%, 10% and 20% *Dialium guineense* fruit pulp inclusion levels compared favourably ($p > 0.05$) with each other. Likewise, rats fed on 20%, 30% and 40% inclusion levels also compared favourably ($p > 0.05$) with each other. For the HDL-C concentration, there was no significant difference ($p > 0.05$) in the concentration of this biomarker in the rats fed on 5%, 10% and 20% of the fruit pulp meal-based diet. In the same way, rats fed on

30% and 40% of the diets also compared favourably ($p > 0.05$) with each other. For low density lipoprotein concentration (LDL-C), triglyceride concentration (TG) and atherogenic index, the fruit pulp meal-based diet at all inclusion levels (5% to 40%) resulted in significant decrease ($p < 0.05$) when compared with the control values but all the inclusion levels compared favourably ($p < 0.05$) with each other in the three parameters.

Table 4: Effect of *Dialium guineense* fruit pulp meal-based diet on serum lipid profile of rats

Treatment	TC (mmol/L)	HDL-C (mmol/L)	LDL –C (mmol/L)	TG (mmol/L)	ATHEROGENIC INDEX
Control	2.77 ± 0.05^{a}	1.94 ± 0.01^{a}	1.08 ± 0.12^{a}	1.78 ± 0.07^{a}	0.61 ± 0.02^{a}
5% *D.g*	3.47 ± 0.06^{b}	2.58 ± 0.02^{b}	0.79 ± 0.03^{b}	0.98 ± 0.09^{b}	0.38 ± 0.03^{b}
10% *D.g*	3.38 ± 0.14^{b}	2.72 ± 0.04^{b}	0.83 ± 0.04^{b}	0.89 ± 0.02^{b}	0.35 ± 0.04^{b}
20% *D.g*	3.95 ± 0.17^{bc}	2.75 ± 0.06^{b}	0.71 ± 0.08^{b}	0.81 ± 0.08^{b}	0.28 ± 0.02^{b}
30% *D.g*	4.19 ± 0.12^{c}	3.32 ± 0.11^{c}	0.69 ± 0.11^{b}	0.84 ± 0.12^{b}	0.27 ± 0.02^{b}
40% *D.g*	4.33 ± 0.33^{c}	3.21 ± 0.21^{c}	0.71 ± 0.13^{b}	0.78 ± 0.02^{b}	0.26 ± 0.08^{b}

Data are means of six determinations ± SEM. Values with superscripts different from the control for each parameter are significantly different ($p < 0.05$). *D.g – Dialium guineense* fruit pulp meal-based diet. TC: Total Cholesterol, HDL-C: High Density Lipoprotein, LDL –C: Low Density lipoprotein Cholesterol, TG – Triglyceride.

The effect of *Dialium guineense* fruit pulp meal-based diet on the organ (liver and kidney) body weight ratios is presented in Table 5. The liver and kidney body weight ratios of the animals maintained on 5, 10, 20, 30 and 40% *Dialium guineense* fruit pulp meal-based diet (all the inclusion levels) were not significantly different ($p < 0.05$) from those maintained on the basal diet (control)

Table 5: Effect of *Dialium guineense* fruit pulp meal-based diet on the % liver- and kidney -body weight ratio of rats

	Organs (ratio in %)	
Treatments	**Liver**	**Kidney**
Control	4.02 ± 0.08^{a}	1.18 ± 0.11^{a}
5%	3.99 ± 0.14^{a}	1.23 ± 0.07^{a}
10%	4.06 ± 0.03^{a}	1.21 ± 0.09^{a}
20%	4.99 ± 0.19^{a}	1.19 ± 0.10^{a}
30%	4.04 ± 0.01^{a}	1.18 ± 0.13^{a}
40%	4.04 ± 0.06^{a}	1.17 ± 0.03^{a}

Data are means of six determinations ± SEM. Values with the same superscript a, across the same column for each organ are not significant different ($p>0.05$). *D.g – Dialium guineense* fruit pulp meal-based diet.

Discussion

Red blood cells (RBC) (erythrocytes) serve as a carrier of haemoglobin. Haemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Ugwuene, 2011; Omiyale *et al*., 2012; Soetan *et al*., 2013; Isaac *et al*., 2013). Thus, the insignificant difference in the red blood cells as well as haemoglobin implies that there will be no reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Ugwuene, 2011; Soetan *et al*., 2013; Isaac *et al*, 2013). This therefore indicates that there will be no traces of anaemia, dehydration, poor diet/nutrition, toxin infection, a malabsorption problem or any other ailment or disorder (Ganong, 2001). Packed Cell Volume (PCV) which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF), is the percentage (%) of red blood cells in blood (Purves *et al*., 2003). According to Isaac *et al*. (2013), Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. As the PCV in the blood of animals fed on *Dialium guineense* fruit pulp meal based diet compared favourably with the control diet, this implies that number of Red Blood Cells and its transportation as well as plasma volume is not compromised. Mean Corpuscular Volume (MCV) measures the size of Red Blood Cells, Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) give the average weight and concentration of haemoglobin in the red blood cell respectively (Ganong, 2001). These three parameters depict blood level conditions (NseAbasi *et al*., 2014). The insignificant difference in the MCV values suggests that the size of the red blood cells is normal because an abnormally small-sized red blood cell will either indicate a failure of the system to synthesize RBCs or lack of available haemoglobin to be incorporated into RBCs to complete the task of synthesis. Infact, low MCHC implies that a unit packed

RBCs contains less haemoglobin than normal and a high MCHC means that there is more haemoglobin in a unit of RBC (Ganong, 2001). Infact, these three parameters with the Packed Cell Volume and haemoglobin are major indices for evaluating circulatory erythrocytes and serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals (Awodi *et al*., 2005; Chineke *et al*., 2006). Therefore, their insignificant differences in this study suggest that there was no alteration in their functions. Overall, the insignificant difference in the RBC (erythrocytes) and indices relating to it (Hb, PCV, MCV, MCH and MCHC) of the rats fed with the experimental diets (5% -40%) throughout the experimental period suggests that the fruit pulp of *Dialium guineense* did not interfere with the metabolism of the component of blood. This might be an indication that there was no destruction of matured RBC's and no changes in the rate of production of erythrocytes (Ganong, 2001). In like manner, there is an indication that the oxygen carrying capacity of the animals was not reduced.

White blood cells (WBC) or leucokyte counts are the total number of white blood cells (Robert, 1976). The main function of the white blood cells (WBC) and its differentials are to fight infection, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune responses (Ganong, 2001). There are a number of types (differentials) of leukocytes that are classified either as granulocytes or non-granulocytes. The granulocytes include neutrophils, eosinophils and basophils while the non-granulocytes include lymphocytes and monocytes (Ganong, 2001). The result obtained from the white blood cell count in this study implies that the experimental diet might not introduce any toxin/infection into the system of the animals as the integrity of resistance to diseases was not compromised (Soetan *et al*., 2013). Although, there were slight variations in the lymphocytes and neutrophil counts in some groups and knowing the fact that these molecules are important in body defence, however, the overall result from white blood cell counts revealed that there was no compromise in the defence system of the body.

Lipids are fatty substances that are normally insoluble in an aqueous environment but are solubilised and transported in the plasma as water-soluble macromolecular complexes known as lipoproteins (Styrer, 1995). Measurement of major lipids can give useful information about the functioning of the heart and are particularly used to estimate increased risk of cardiovascular disease (Naik, 2012). Although, significant increase in total cholesterol can indicate a high risk of cardiovascular disease, the significant increase in the total cholesterol concentration of the all the groups fed on the fruit pulp meal based diet may not be fully attributed to increased mobilization of free fatty acids from the peripheral fat depots since there was no concomitant increase in the low density lipoprotein (LDL) and triglyceride concentration in all the experimental groups and this might be justified by the increase in high density lipoprotein (HDL) cholesterol (Aguilar *et al*, 2011). Increased LDL concentrations are associated with atherosclerosis, heart attack, stroke and cardiovascular diseases (Bordia and Verma, 1998; Cromwell and Otvos, 2004) and could enhance obesity. *Dialium guineense* fruit pulp meal based diet reduced LDL susceptibility to oxidation and possibly increased the resistance of plasma LDL to oxidation. This could therefore prevent obesity (Ijeh *et al*., 2015). This might be due to the presence of flavonoids and other phenolic constituents in the fruit pulp (Oyegoke and Oladiji, 2014) as these free radicals scavenging secondary metabolites help prevent cardiovascular diseases by interfering with the oxidation of the very low density lipoproteins and low density lipoproteins, which are the chief engineers of atherosclerosis (Omodamiro and Nwankwo, 2013). Dietary incorporation of the fruit pulp of *Dialium guineense* also decreased the triglyceride concentrations, and could therefore improve hypertriglyceridemia and the associated obesity (Ijeh *et al*., 2015). High Density lipoprotein are referred to as the "good cholesterol" since they remove cholesterol to the liver for excretion, therefore their significant increase will be appropriate for the increased total cholesterol and thus reduce the risk of coronary artery disease. Atherogenic index serves as a direct pointer to exposure to atherosclerosis which is a precursor of all cardiovascular diseases (Oloyede *et al*., 2015). High atherogenic index may suggest that atherogenic plagues have been deposited on the walls of the arteries (Rosalki, 1967; Sartej *et al*., 2012) and these may lead to partial or complete blockage to the flow of blood in the arteries exposing the individual to all forms of cardiovascular diseases (Oloyede *et al*., 2015). In this study, since the computation for the atherogenic index reveals that there is no group in which the index was greater than 5 (Ng *et al*., 1997), it can be inferred that the diet does not possess positive risk for atherogenesis. The fruit pulp probably plays atherogenic role through the inhibition of lipid oxidation and therefore could promote the reverse cholesterol transport pathway (Ijeh, 2015). This can be evidently supported from the result of high density lipoprotein concentration in this study. Oyegoke and Oladiji (2014) reported the presence of saponins and fibre in the fruit pulp of *Dialium guineense*. Saponins have been reported to reduce the uptake of cholesterol at the gut through intra-lumenal physicochemical interaction (Price *et al*., 1987). Likewise, fibre promotes a sense of satiety which helps to prevent overeating and weight gain (De Moura *et al*., 2009).

Furthermore, dietary fibres slow down the rate of absorption of cholesterol in the gastrointestinal tract. Therefore, the serum lipid modulating property of this fruit pulp may therefore be attributable to its saponin and fibre content.

Analysis of organ weight in toxicology studies is an important endpoint for identification of potentially harmful effects of test compounds/ toxins (Bailey *et al*., 2004). Organ body weight ratio is a marker of cell constriction and inflammation (Moore and Dalley, 1999). In some cases, the organ might shrink as a result of excess loss of fluid due to some abnormal conditions. At other times, the organ might swell abnormally suggesting excessive fluid retention, which might be due to osmotic pressure. The lack of significant change in the size of the liver and kidney relative to their entire weight of the animals suggest that the fruit pulp meal-based diet of *Dialium guineense* did not cause any inflammation or constriction in the cells of the organs investigated.

Conclusion

Overall, although there are some variations in the values of some haematological parameters, nevertheless, the present findings have shown that the fruit pulp of *Dialium guineense* is not likely to produce any severe toxicological effects on the haematological parameters, lipid profile and on organ weights when given at the experimental inclusions.

Conflict of Interest

The authors declare that there are no conflict of interest arising from this research.

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Sero-prevalence of HIV-1 Among Commercial Sex Workers in Abuja, Federal Capital Territory, Nigeria

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Abstract

The role of Commercial Sex Workers (CSWs) in facilitating transmission of sexually transmitted infections (STIs) including HIV is a subject of continued interest. Sex for money has varied structures across the world usually found in organized and semi-organized type of residence and in some cases as freelance. This study was aimed at determining the prevalence of HIV-1 among CSWs in Abuja, FCT, Nigeria. A non-randomized, non-bias study was conducted among 400 CSWs (age brackets 16-50 years) who voluntarily consented to participate in the study. The recruitment was done in brothels, hotels and in settlement camps among 143 male sex workers (MSWs) and 257 female sex workers (FSWs). The test was carried out using Uni-Gold HIV 1/2 test kit (Trinity Biotech, Ireland) and Serocard HIV 1/2 test kit (Trinity Biotech, Ireland) using serial algorithm. 124(31%) of the CSWs tested were HIV antibody positive. Based on gender, 25(17.5%) of the MSWs were positive while 99(38.5%) of the FSWs were tested positive. Considering the age of the volunteers, those within the age brackets of 31-35 years has the highest prevalence of 41(32%) of the total positives. Male sex workers had the highest prevalence within the age bracket of 31-35 years with 9(36%) positive while the FSWs within the age brackets of 26-30 years has the highest prevalence of 35(35.4%). This study indicated that there is epidemics of HIV-1 among CSWs but higher among the FSWs. It is therefore concluded that commercial sex workers are major source of heterosexual transmission of HIV-1 in Abuja.

Keywords: CSWs, HIV, MSWs, FSWs, Prevalence

Introduction

The role of Commercial sex workers (CSWs) in facilitating transmission of sexually transmitted infections (STIs) including HIV is a subject of continued interest (Josephat *et al*., 2012). Sex for money occupation has varied structure across the world. This group of people who sell sex are found in organized or semi-organized and in some cases as freelance. In organized (eg, pimp, manager) through establishments such as bars, brothels, or saunas, or in more public spaces such as parks, streets and festivals. Additionally, a growing portion of sex work is arranged through the internet (UNAIDS, 2009; Logie *et al*., 2017). Most sex workers worldwide are women; however, substantial populations of male and transgender sex workers exist in many countries (van Veen *et al*., 2010).

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The dynamics of HIV transmission among male and transgender sex workers could be further complicated by the heightened biological risks of anal intercourse, high prevalence of HIV in some subgroups of MSM, and the large proportion of male and transgender sex workers who report bisexual practices. (Stefan *et al.,* 2012).

Sexual transmission accounts for a majority of cases of human immunodeficiency virus (HIV) infection worldwide (Maria *et al*., 2006). Sexually transmitted infections (STIs) other than HIV have been found to enhance the transmission of HIV and to be more prevalent among female commercial sex workers (FSWs) (Mwumvaneza *et al*., 2017). Behaviours such as multiple sex partners, irregular condom use by clients, and co-infection with other STIs constitute potential risk factors associated with HIV infection among CSWs (Maria *et al*., 2006) Commercial sex workers are therefore, considered a risk group for heterosexual spread of human immunodeficiency virus (HIV) worldwide (van Veen *et al*., 2010). Regular partners or non-commercial partners of the female sex workers (FSWs) are another important risk group. As such, FSWs are often categorised among the populations "most-at-risk" to HIV due to behaviours that heighten their exposure to the virus (Josephat *et al*., 2012). Talbott, (2007) opined that the number of HIV-infected sex workers in any country is highly significant for explaining the HIV prevalence levels across countries.

UNAIDS estimates that over 33 countries including 22 sub-Saharan African countries have achieved declines in HIV incidence of over 25% (UNAIDS, 2010). The decrease in incidence of HIV in sub-Saharan Africa can be attributed to advancement in global fight against the infection but could mask sustained or expanding spread among populations who are most at risk including sex workers, men who have sex with men (MSM), and intravenous drug users (IDUs). HIV prevalence among female sex workers in sub-Saharan Africa varies between 21% and 75% (Bimal *et al.,* 2013) and sex work is assumed to have had a significant impact on the spread of the infection in this area (van Veen *et al.,* 2010). HIV prevalence among Female Sex Workers in India at national level were from 5.0% in 2007 to 2.2% in 2015 (NACO, 2016). Understanding the dynamics of HIV infection in this key population is critical to developing appropriate prevention strategies for the control of HIV in the sub-region. The aim of this study is to describe the prevalence of HIV among Commercial Sex Workers in Federal Capital Territory Abuja, Nigeria.

Materials and Methods

Study Area

This research work was conducted in Abuja, the Federal Capital of Nigeria. Abuja is located at the centre of Nigeria with land mass of 8,000 square meters. It has boundary with Kaduna in the North, Plateau State in the East and South East, Kogi State to the South West and Niger State to the West. It is divided into six administrative area councils; Abaji, Kwali, Gwagwalada, Bwari Kuje and Municipal area councils. Abuja climate is regarded as favourable throughout the year which further encourage the influx of the people. Because of this migration there are lots of social interactions among the people of sexually active group (Henry, 2008). This interaction predisposes so many people to high risk factors that aid HIV transmission.

Study Population

Four hundred volunteer commercial sex workers in hotels, brothels and in settlement camps in Abuja were recruited for this study. They were counseled and those that voluntarily consented were recruited for the study. Community based recruitment was done at Nyanya, Kado, Mabushi among commercial sex workers while the brothel and hotel recruitment were done at Nyanya, Kubwa Zuba and Gwagwalada. The recruitment was non-randomised without bias of sex and questionnaires were administered on those that consented to participate in the study.

Demographic information was collected using questionnaires. For the purposes of this study, the information collected from the participants includes age, sex, occupation and residential address.

Ethical Consideration

Ethical clearance for the study was sought from the Institutional Review Board of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu–Abuja, Nigeria in

accordance with the code of ethics for biomedical research involving human subjects. The participants were sufficiently counseled and informed consent obtained with the assurance that all information would be treated with utmost confidentiality during and after the study.

Sample collection

Sample collection was done by finger pricking according to the standard practice. Briefly this was done by choosing a finger and swabbing the tip of the finger using cotton wool soaked in 70% alcohol. The alcohol is allowed to dry off and a disposable lancet was used to pierce the finger deep enough to allow adequate flow of blood. Two capillary tubes were filled with blood and used for the test.

Sample Analysis

All the four hundred samples collected were tested for HIV-1 antibody using Unigold™ (Trinity Biotech, Ireland) and Serocard HIV1/2 (Trinity Biotech, Ireland) HIV Test kits. All the test was carried out according to the manufacturer's instructions using serial algorithm. The test was carried out with Unigold™, and those that nonreactive to HIV antibody are reported as negative while those that are reactive with the Unigold kits are repeated using Serocard HIV-1/2 Antibody Test kit. For the purpose of quality assurance every tenth negative sample was repeated with Serocard. The reactive samples were reported as positive, while the non-reactive were reported as negative.

Statistical Analysis

All statistical analyses were performed using Statistical Product and Service Solution (SPSS) software (version 17.0, SPSS, Chicago, USA). Prevalence was expressed as percentages and the level of significance was set at $p < 0.05$.

Result

Out of four hundred commercial sex workers tested, 124(31.8%) were HIV-1 positive (Figure 1). The volunteers were segregated by gender and 143 were males while 257 were females. The male population consist of men who have sex with men, hotels and brothel workers who are regular customers of the female commercial sex workers. 25(6.25%) of the males were also positive to HIV-1 antibody, while out of the 257 female sex workers tested, 99(24.8%) were positive. There was significant difference between the prevalence of HIV-1 among the male and female sex workers ($p \leq 0.05$). A total of 124(31%) of the commercial sex workers tested were positive. The prevalence of HIV-1 among commercial sex workers was statistically significant ($p \leq 0.05$).

The result was disaggregated by age bracket; HIV-1 prevalence was highest among the respondents in the age bracket of 31-35 years of age with prevalence of 6.5%, followed by those in those within the age bracket of 21-25 years with prevalence of 6.25%. The result also showed that respondents in the age bracket of 36-40 has prevalence of 3.25% and 16-20 and 41-45 years both had prevalence of 2.25% each while those within the age bracket of 46-50 years had the least prevalence of 0.25%. Analysis showed that there is no significant difference between the age brackets of 26-30 and 31-35 years while there was significant difference between these groups and within the age brackets of 16-20, 36-40, 41-45 and 46-50 years ($p \leq 0.05$) (Table 1).

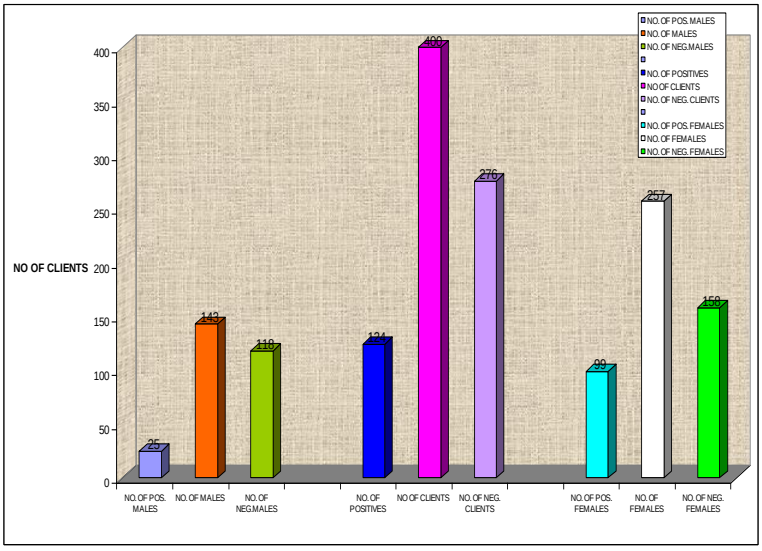

Figure 1: Distribution of HIV-1 among Commercial Sex Workers in Abuja.

Table 1: Prevalence of HIV-1 among CSW in Abuja disaggregated by age brackets.

Age Brackets (Years)	Number of Volunteers	Number of positive volunteers
16-20	47	9 (2.25%)
21-25	93	25 (6.25%)
26-30s	102	41 (1.5%)
31-35	75	26 (6.5%)
36-40	37	13 (3.25%)
41-45	25	9 (2.25%)
46-50	21	1 (0.25%)
Total	400	124 (31%)

Table 2 represents the prevalence of HIV infection among commercial sex workers disaggregated by gender and age. The result of this study showed that the rate of infection is higher in females than among the males and the analysis showed that there was significant difference between the rate of infection among the male and female commercial sex workers ($p \leq 0.05$). The males within the age range of 31-35 had the highest prevalence while the females in the age range of 26-30 had the highest prevalence.

Table 2: Distribution of HIV-1 among CSW in Abuja disaggregated by gender and age.

Age Brackets	Number of Male Sex Workers		Number of Female Sex Workers	
	No. of Respondents	No. positive MSW	No. of Respondents	No. positive FSW
16-20	9	1 (0.25%)	38	8 (2%)
21-25	27	1 (0.25%)	66	24 (6%)
26-30	33	6 (1.5%)	69	35 (8.75)
31-35	27	9 (2.25%)	48	17 (4.25%)
36-40	17	4 (1.0%)	20	9 (2.25%)
41-45	16	4 (1.0%)	9	5 (1.25%)
46-50	14	0	7	1 (0.25%)
Total	143	25 (6.25%)	257	99 (24.75%)

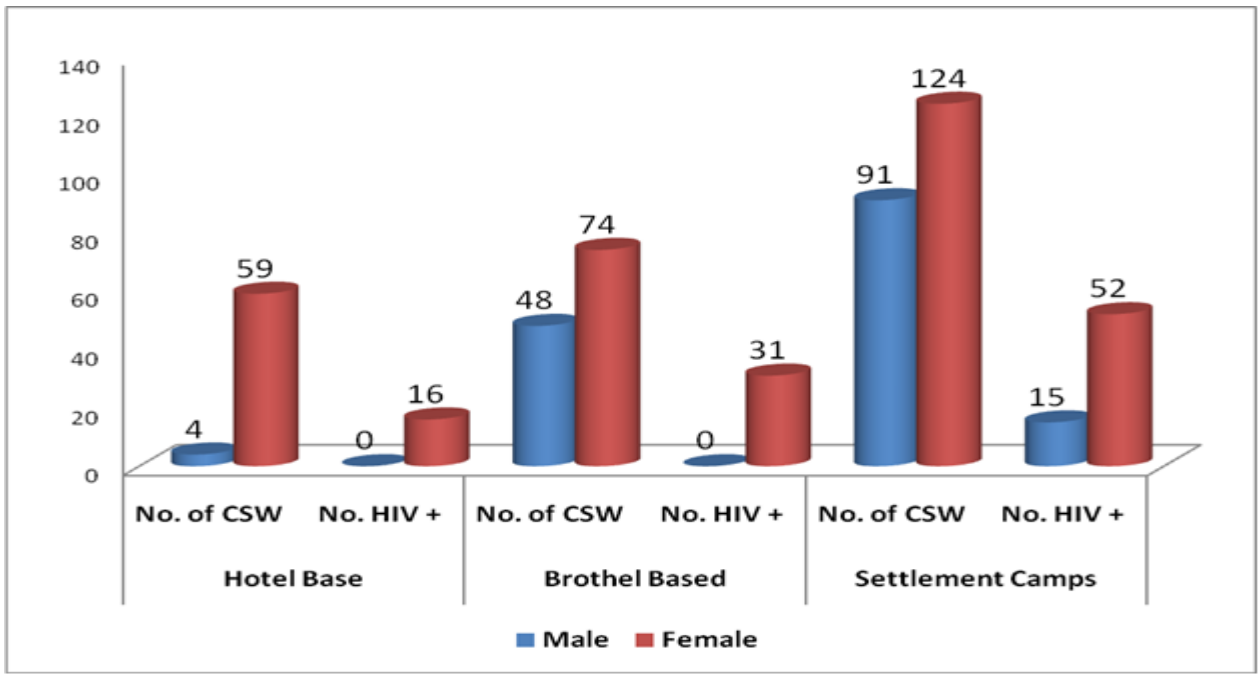

Figure 2: Prevalence of HIV-1 AMONG commercial sex workers according to type of residence.

Commercial sex workers were disaggregated by gender and compared according to the class of residence. In all the classes, the females were more infected. Among those residing in hotels 16(4%) of the females were positive to HIV-1. The brothel based female sex workers had 31(7.75%) HIV-1 prevalence while 13% of the females residing in settlement camps were positive. There was no infection recorded among those residing in hotel and brothel while 3.75% of the males tested at the settlement camps were HIV-1 positive (Figure 2).

Discussion

In the developing world, commercial sex workers (CSW) and their clients have been well identified as core groups that play a major role in the propagation of sexually transmitted diseases (STDs) and HIV (Michel *et al*., 2002). The findings of this study of CSWs in Abuja the Federal Capital Territory of Nigeria indicate that there is HIV epidemics among this vulnerable group. The prevalence of 31% among commercial sex workers in Abuja though lower, is comparable to the result of a study of 51% prevalence among commercial sex workers in Rwanda by Mwumvaneza *et al*. (2017). Also a study in Senegal reported prevalence of 19.8% (Wang *et al*., 2007). This is however much higher than the result of the study by van Veen *et al*. (2010) where the prevalence of HIV among sex workers was 5.7% in Netherlands. According to the criteria of the Joint United Nations Programme on HIV/AIDS, the HIV epidemics in this group appear to have transitioned from low level to concentrated, because overall HIV prevalence among CSWs in Abuja is >5%. The study suggested significant roles played by sex workers in the rapid transmission of heterosexual HIV infection among Nigeria populace.

Although female sex workers have long been understood to be a key affected population, the scope and breadth of their disproportionate risk for HIV infection had to date not been systematically documented. The result of this study also indicated that the prevalence is higher among female sex workers than the male with prevalence of 6.25% among the male sex workers and 24.8% among the female sex workers. The prevalence of HIV among female sex workers in this study is higher than the findings of other studies by Thomas *et al*. (2008); Adithyan *et al*. (2017). These results are also higher than the 9% reported prevalence among the sex workers in Jamaica by Hope Enterprises (2005) and 4.9% by Jacqueline, (2014). The 2011 systematic review and meta-analysis on low and middle income countries found an overall HIV prevalence in FSWs of 12% and an odds ratio for HIV infection of 13.5% compared to other women of reproductive age (Baral *et al*., 2012). 24.8% among FSWs in this study agreed with the findings

that HIV prevalence among female sex workers in sub-Saharan Africa varies between 21% and 75% (Bimal *et al*., 2013) and sex work is assumed to have had a significant impact on the spread of the infection in this area (Cote *et al.*, 2004). In Western Europe, the prevalence of HIV in female CSW is generally below 2%, except for those who are injecting drug users (IDU) (Eurohiv, 2002). Higher HIV rates are also found in transgender CSW, ranging from 14 to 74% (Belza, 2005). Our result is in consonant with the result of van Veen et al., 2010, where the prevalence of HIV among the transgender was 18.8%, while it was 1.5% among the female sex workers. The finding of this study of 6.25% among the male sex workers however is very low when compared to the work of Figueroa, (2015), that reported HIV infection rate of 52.9% among the male sex workers. It was reported that HIV infection is 9-fold higher among transgender sex workers than among non-transgender female sex workers (UNAIDS, 2014). A systematic analysis review findings suggest transgender women who are sex workers have significantly higher HIV infection rates in comparison with transgender women not involved in sex work (Operario *et al*., 2008).

On considering the age brackets of the sex workers, the prevalence was found to be higher among the youth which is contrary to a report from India where the prevalence is higher among the older sex workers which is presumed to be as a result of level of exposure (NACO, 2016). Among the male sex workers, the prevalent was highest from the people in the age brackets of 31-35 years which can be best explain by the fact that they are the most economically viable class among the sexually active stage. The highest prevalence among the female sex workers in this study was higher among the people within the age range of 21-30 years which are the most active sexual stage. It can be postulated that this class may be receiving high patronage because of their youthful age and may be very well exposed to the infection because of lack of adequate knowledge of transmission of the virus.

The sex workers in the settlement camps were most affected in both the male and female sexes. Though from this study, there were more volunteers from the settlement camps than the brothel and hotels, the rate of infection may be attributed to lifestyle and the level of awareness about HIV transmission. The result indicated that the better organized the sex workers are, the lower the rate of infection. The result of this study showed there is higher of HIV among females than males from the settlement camps. Prevalence of 3.75% and 13% among male and female sex workers in the settlement camp was recorded respectively. The trend is similar to that of Mafigiri *et al*., (2017) where the prevalence was higher among the female sex workers in settlement camps (25.9%) than the male sex workers (12%) in Uganda.

The findings from this study have indicated that commercial sex workers are major source of heterosexual means of transmission of HIV/AIDS in Abuja. It is therefore recommended that for effective preventive and control measures, policies that will enhance the preventive measure of vulnerable population group like commercial sex workers should be developed and implemented immediately. It is also recommended that this type of survey to determine the sero-prevalence, knowledge of attitude and practice to HIV/AIDS should be conducted at time intervals. These will help in mitigation in the spread of the dreaded HIV/AIDS disease.

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EFFECTS OF *ALOE VERA* (L.) BURN F. EXTRACT ON *ASPERGILLUS FLAVUS* AND *ASPERGILLUS NIGER* ISOLATED FROM GROUNDNUT (*Arachis hypogea* L.)

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Abstract

Arachis hypogeal is a species of flowering plant in the family Fabaceae. It is infected by *Aspergillus flavus* and *Aspergillus niger* in all stages of growth, storage and in transit. These fungi cause spoilage, loss by contaminating seeds; cause aflatoxin contamination which are carcinogenic and hepatoxic. Groundnut infected by these fungi may cause aspergillosis in immune-compromised individuals. In this study the antifungal efficacy of unfractionated whole *Aloe vera* extract on *A. flavus* and *A. niger* isolated from healthy and deteriorated groundnuts (obtained from New Benin and Oba Markets (Ring Road) was investigated. Bioassay of the *Aloe vera* was done by the pour plate method. The results showed total inhibition of the *A. flavus* isolated from healthy groundnut at 100, 75, 50 and 25% concentration respectively, for New Benin market; a high inhibition of the *A. flavus* isolated from the deteriorated New Benin groundnut at the 100 and 75 % concentration respectively and a moderate inhibition at 50 and 25%. There was no significant inhibition at all concentrations of the *A. niger* isolated from the healthy groundnuts for Oba market. Phytochemical analysis of the *A. vera* used showed the presence of Alkaloids, phenols and Tannins which validates the antifungal properties of *A. vera.* The negative result obtained in the bioassay against *A. niger* may be that the concentration of *A. vera* was not sufficient to cause an effective inhibitory action against its growth. Further work is ongoing to determine the extract concentration that would effectively inhibit *A. niger*.

Keywords: *Aloe vera*, Phytochemicals, *Aspergillus flavus*, *Aspergillus niger*, *Arachis hypogea*

Introduction

Naturally occurring biochemical compounds (Phyto-chemicals) of plants which are obtained by chemical or mechanical extraction are known as Plant extracts (Okungbowa *et al*., 2012). These extract contribute a great deal in opposing the pathogenicity of microorganism (Vyvyan, 2002).

Aloe vera is a member of the liliaceae family. *Aloe* species numbers over 300 of which *A. vera* L. is the most widely recognized and utilized for various medical and cosmetic purposes (Surjushe *et al*., 2008; Joseph *et al*., 2010).

A bitter yellow liquid exudates of high phenolic content and a mucillagenous pulp are the main parts obtainable from the leaves of *A. vera*. The liquid fraction constituents are largely phenolic in nature (Reynolds, 1985). Carbohydrate polymers such as glucomannans and pectic acid including some other organic and inorganic content can also be obtained from the pulp. Newall *et al.* (1986) reported that *A. vera* contains some phytochemicals such as saponins, tannins and other substance such as mono and polysaccharides, sterols, organic acids, enzymes, vitamins and minerals.

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Groundnut native to South America, Mexico and Central America is an herb grown annually (Rashmi *et al*., 2017). In Nigeria, other than palm oil, it is one of the most important and generally accepted source of cooking oil compared to other plant sources. Most farmers and agro-merchants store groundnuts in time of excess to resale at the time of shortage. However, these stored legumes get infected by some species of the genus aspergilla which produces a wide range of metabolite, one of such is Aflatoxins.

A good deal of fungi which may be identified by fungal spores: that give characteristic coloration, inhabits soil and many of them infect and damage the seed and germinating seedlings of groundnut. Nuts are susceptible to contamination by *A. flavus* and *A. niger* during the pre and post storage phase and on transit. In groundnut, fungi infect the leaves, seedlings and cause rots of seeds thereby causing diseases. Some of the diseases of the seedlings caused by these fungi are: root rot, stem rot, wilts, blight and pod rot; that of the leaves are: rust, early and late leaf spots

Aspergillus niger has been implicated to be pathogenic in studies carried out by Sharma, (2012). It causes the Crown rot of peanuts which is characterized by root curling and deformation of the upper parts of the plant. *Aspergillus niger* is also reported to produce ochratoxin A, fumonisin B2 and aflatoxins (Abraca *et al*., 1994; Schuster *et al*., 2002; Noonimabc *et al*., 2009; Al-Abdalall, 2009) in stored commodities. The fungus, *Aspergillus flavus*, produces aflatoxin and causes aflaroot or yellow mold of groundnut. Affected seeds shrivel up, dry and are covered by spores that are yellow or greenish in colour. The cotyledons become necrotic with margins that are reddish brown; seedlings are highly retarded in growth, the leaves are pale and light green in colour with a reduced size (Pal *et al.*, 2014).

Antibiotics, antibiosis and chemicals have been used to treat *Aspergillus niger* infections. However, Biological control has been found to be the best and most effective treatment (Sharma, 2012). In this study, the antifungal potentials of unfractionated extract of *Aloe vera* on *A. niger* and *A. flavus* isolated from fresh and deteriorated groundnut was investigated and the extract concentrations that can inhibit the mycelia growth and development of this species was determined.

Materials and Method

Preparation of media

39 grams powder of Potato Dextrose Agar (PDA) in one liter of deionizer water was soaked for 10mins according to standard laboratory protocol. The mixture was swirled to mix and sterilized at 121^{o}C for 15 minutes; it was then allowed to cool to 47^{o}C and mixed well before pouring into Petri dishes.

Isolation of micro organism

Tissue maceration method was used to isolate organisms and stock solution prepared. Cultures were obtained by using the serial dilution and pour plate method; Cloramphenicol at 0.02 mg per 200ml of medium was introduced at pouring to inhibit the growth of bacteria. Sub-culturing of the isolates was done; pure isolates obtained was streaked into nutrient agar slants in a McCartney bottle and stored in the refrigerator at 4^{o}C. The isolated fungi were identified using the criteria adopted by Raphel and Fennel 1965; Samson *et al*., 2002.

Preparation of Plant extract

The *A. vera* was oven dried at 50^{o}C. A soltex extractor was used to extract the gel which was then concentrated with a rotary evaporator

Phytochemical analysis of Plant extract

The detection of the phytochemical present in the *Aloe vera* extract was done using the official methods of analysis. (A.O.A.C, 2005)

Bioassay of Plant extract

Spore suspension of the pure cultures of the isolates was prepared by using a flamed wire loop to scoop a portion of the pure isolates into 4mls of water that was sterilized at 121^{o}C for 15 minutes to dislodge the spores into the sterile water. 0.5mls each of the spore suspension was then pipetted with a 5mls syringe into Petri dishes which were labeled New Benin 100%, New Benin 75%, New Benin 50%, New Benin 25% and New Benin control. This was also repeated for all the isolates obtained from Oba market respectively in duplicate. 1ml of the plant extract was then pipetted into each of the spore suspension in the Petri dishes, this was then followed by PDA using the pour plate method. The Petri dishes were then kept at room temperature for 5 days.

RESULTS

Table 1: Percentage composition of the phytochemicals present in *Aloe vera* used for this research work

Phytochemicals	**Percentage composition (%)**
Tannins	0.54
Saponins	0.011
Flavonoids	0.44
Alkaloids	0.66
Phenols	0.32

Table 2: *Aspergillus* species isolated from *Arachis hypogea* isolated from groundnut obtained from two locations.

Locations	Morphological description	Suspected organisms
New Benin Market (deteriorated)	Black mycelia growth	*Aspergillus*
	Light green mycelia growth	*Aspergillus*
New Benin Market (fresh)	Black mycelia growth	*Aspergillus*
	Light green mycelia growth	*Aspergillus*
Oba Market, Ring road (deteriorated)	Black mycelia growth	*Aspergillus*
	Light green mycelia growth	*Aspergillus*

The isolated fungi were identified using the criteria adopted by Raphel and Fennel 1965; Samson *et al*. (2002).

Discussion

Groundnut is an important food crop and oil seed in Nigeria. it is one of the crops that contributes to the gross domestic product (GDP) of the country: Nigeria is the fourth largest producer in the world and the highest producer in Africa with 1.55 million metric tons (*FAOSTAT*, 2014). World trade in groundnut has declined since the 1990′s, reason being that there has been a decline in the export from developing countries. (FAO, 2002; Revoredo and Fletcher 2002; Sowley, 2016)

Aflatoxin contamination of groundnut and its by-product is a culprit for these decline: standard for aflatoxin content has been set by the main importers and these standards are often not met by developing countries (Sowley, 2016). Research is being carried out to discover the best method of forestalling contamination from aflatoxin in food crops; the use of biocontrol with the utilization of phytochemicals from plant extract is one major area which is critically being looked into.

The antimicrobial potentials of most plants can be attributed to the phytochemicals imbedded in them. These phytochemicals are secondary metabolite used by these plants to protect themselves from predators; they also have diverse use in medicine and pharmaceutics. The presence of phenol, lignin and saponins confers antifungal properties on plants whose extract has been used as biocontrols.

Several authors have investigated the efficacy of plant extract and essential oils as antifungal agent. Iraj *et al.* (2006) investigated the inhibition of growth and the morphological alterations of *A. niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock* and reported that the essential oils from these plants could be safely utilized as a preservative. Sitaria *et al*. (2011) investigated the antifungal efficacy of *A.vera* gel against plant pathogenic fungi and concluded that the gel of *Aloe* completely inhibited the growth of *Drechslera hawaiensis* and *Alternaria alternate.*

In this present investigation, the in-vitro antifungal efficacy of *A. vera* on *Aspergillus flavus* and *Aspergillus niger* was investigated and results showed that the *Aloe vera* extract significantly inhibited the growth of *A. flavus* at 100% and 75% with moderate inhibition was recorded at 50% and 25% concentration compared to the control. There was a significant inhibition of all the *A. flavus* isolate compared to the *A. niger* in which there was no significant inhibition at all concentration. The insignificant inhibition of the extract against *A. niger* correlates with the result of Sitara *et al*. (2011) who reported that *Aloe vera* possess remarkable antifungal activity towards all fungi compared to the control except *A. niger* when the antifungal activity of *Aloe vera* gel against plant pathogenic fungi viz *A. niger*, *A. flavus*, *Alternaria alternate*, *Drechlera hawaiensis* and *Penicillium digitatum*. The extract showed complete inhibition at all concentration on the *A. flavus* isolated from fresh groundnut than the isolates from deteriorated groundnut. The presence of the fungi in the fresh groundnuts may be that before harvest and /or during transits, some of these groundnuts could have been infected; the high efficacy of the extract on the isolates of the fresh than the deteriorated groundnut may be that the infection of the pathogenic fungi is more severe in the deteriorated state than in the fresh groundnut. The extract can be tested as a coating to prolong the time of spoilage of the groundnut just as been done by Misir *et al*. (2014) where *Aloe vera* gel was used as a novel edible coating for fresh fruit. *Aloe vera* can also be used as a preservative, just as been done by Serrano *et al*. (2006) where grapes at 1^0C coated with the gel of *Aloe* could be preserved for 35 days against 7 days of untreated grapes. All the plates in which there was a positive result on the effect of the extract was kept at ambient temperature for an extra 7 days in addition to the 7 days from which the result were taken, it was

observed that the plates looked the same as that of the result taken the previous week.

CONCLUSION

The result obtained from this present study confirms the antifungal efficacy of *Aloe vera* extract against *Aspergillus flavus* isolated from *Arachis hypogea*, while there was no inhibition of the *A. niger* isolates at all concentration: the inhibition of the *A. flavus* isolates at both locations may be that the concentration of the *A. vera* extract was sufficient to inhibit its growth and not sufficient to cause an effective inhibitory action against the growth of *A. niger*.

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Antibiotic Susceptibility Studies of *Pseudomonas aeruginosa* Isolated from Wounds of Patients Attending General Hospital Minna, Nigeria

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Abstract

This work was carried out to determine the antibiotic Susceptibility profile of *Pseudomonas aeruginosa* isolated from wounds of patients attending General Hospital Minna Niger State. A total of 100 wound swab samples were collected from July to September, 2015 and subsequently analyzed. Isolation and identification of *Pseudomonas aeruginosa* was carried out using Cetrimide agar and subjected to series of biochemical tests. Antibiotic susceptibility tests were carried out on the isolates. One way Anova was used to statistically analyze the data. The bacteria isolated and identified from patient wounds includes, *Pseudomonas aeruginosa*, 53(86.88%), *Pseudomonas fluorescens*, 6(9.83%), and *Pseudomonas* sp, 2(3.29 %). *P. aeruginosa* showed susceptibility to Ciprofloxacin (94.3%), Tarivid (86.8%), and Augmentin (73.5%), but resistant to Streptomycin and Ampicillin. *P. fluorescens* exhibited Susceptibility to Ciprofloxacin (83.3%) and showed 100% resistance to Gentamicin, Streptomycin, Septrin and Ampicillin. The *Pseudomonas* sp was Susceptible to Tarivid by 100%, and resistant to Reflacin, Streptomycin, Septrin and Ampicillin by 100%. The statistical analysis revealed that Age, Occupation, Cause of wound and Educational status of the patient had significant effect on infection with *Pseudomonas aeruginosa* ($P<0.05$). The presence of *Pseudomonas aeruginosa* in wounds could lead to the degeneration of such wounds, and thereby causing delay in the healing of the wounds.

Key words: Isolation, *Pseudomonas aeruginosa*, wounds, patients.

INTRODUCTON

Contamination of wound by bacteria is one of the most important cause of mortality and morbidity, novel nosocomial pathogens and antibiotics resistance leads to reviewing susceptibility patterns of several organisms isolated from wounds, one of which is *Pseudomonas aeruginosa.* (Mehta *et al.*, 2007). Most of the time, development of wound infections is largely dependent on two factors which are; impairment of host natural defence system and systemic dissemination of colonizing organism (Adebayo *et al.*, 2003). Wounds are majorly classified as accidental which can be deep or superficial, pathological which can be wound from diabetic foot (diabetes mellitus), ulcer, abscess, and post-operative wounds also known as surgical wounds (Bowler *et al.*, 2012). Other wounds can be due to animal bites (snakes, dogs etc.). Whatsoever the nature of the wound may be, infection in wound is caused by attachment of microorganism to host cells where they proliferate, colonize and become better adapted to cause damage to the host tissues (Collier, 2003). So many variety of microorganisms have the ability to infect wounds and they range from bacteria to fungi and parasites. Constant widespread use of antibiotics has led to the emergence of resistant pathogens which has contributed immensely to mortality and morbidity. (Nwachukwu *et al.*, 2009). It has also been discovered that wound infections can be found world over, but the organism will usually vary with the geographical area, the normal flora resident on the skin and clothing at the site of wound (Anupurba *et al.*, 2010).

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In general, wounds can be said to be infected with bacterial cells if pus-like liquid emanates from it, even without culturing to determine positive result. Many wounds are usually colonized by bacteria even if they are not infected. Culturing of some wound samples may not yield pathogens, and this is because some of these organisms are fastidious in nature i.e. special nutrient required for their growth (Nwachukwu *et al.,* 2009). Infections in wound can be caused by many bacterial agents. Injury to the skin leads to coagulation and acute inflammatory response which is followed by exposure of inner skin tissues (subcutaneous) which will subsequently bring about loss of skin integrity and it will provide moist, warm nutrient and conducive environment for microbial proliferation or multiplication. Although there has been an introduction of a wide variety of antibiotics and antimicrobial agents with anti-Pseudomonas activity. There has been an incessant increase in the activity of *Pseudomonas aeruginosa* in wounds and it contributes to morbidity and mortality in hospitalized patients (Nwachukwu *et al.,* 2009). *Pseudomonas aeruginosa*is a gram negative rod shaped organism that has ability to resist antimicrobial agents and that is the main reason it can be found in wounds and nosocomial infections. It can colonize a wound rapidly and this makes it very difficult to deal with and it will usually make antimicrobials ineffective (Nagoba *et al.,* 2009).

MATERIALS AND METHODS

Sample collection: A total of 100 wound samples were collected using swab sticks from, both admitted patients and out-patients that attended the General Hospital, Minna. Questionnaire was administered to assess social demographic factors of patients before sampling. Following laboratory rules of sample collection, surface of wound was first cleaned with normal saline in order to exclude indigenous microbial population, and to reduce microbial load (Cheesbrough, 2006).

Isolation of *Pseudomonas aeruginosa* from infected wound samples.

Collected samples were collected using sterile peptone water and transported to the laboratory where a sterile wire loop was used to inoculate samples onto solidified cetrimide agar, which is selective for the isolation of *Pseudomonas* from infected samples. Plates were incubated for 24hrs at 37^0C, after which plates were observed for cultural characteristics of *Pseudomonas aeruginosa* isolates (Cheesbrough, 2006)

Biochemical characterization of the isolates.

Suspected pure colonies were subjected to different biochemical tests after gram staining according to Cheesebrough (2006) for proper identification. The biochemical tests include catalase, coagulase, oxidase, motility, urase, indole production, methyl red, Voges-Proskauer, citrate utilization and sugar fermentation.

Antibiotics Susceptibility Testing

This test was carried out to determine the susceptibility profile of the isolates to some therapeutic agents used in hospitals. It was performed on Mueller-Hinton agar using the standard disc diffusion method recommended by the National Committee for Clinical Laboratory Standards, by preparing the 0.5 McFarland's standard to standardize the turbidity of the isolates' suspension. The suspension of the Pseudomonas isolates were prepared from overnight incubated isolates on the Nutrient agar slants, which were later inoculated evenly on the surface of sterile Mueller-Hinton agar plates using sterile swab sticks, after which multi discs antibiotics were placed on the surface of inoculated plates and incubated at 37°C for 24 hours. Reading and measuring of zones of inhibition was done in millimeters. Isolates were ranked Susceptible or resistant by comparing the zones of inhibitions with the values that are recommended on the standard charts (CLSI, 2012).

Statistical Analysis

One-way Anova was used to determine the association between the social demographic factors and the occurrence of *Pseudomonas aeruginosa* in the wounds patients.

RESULTS

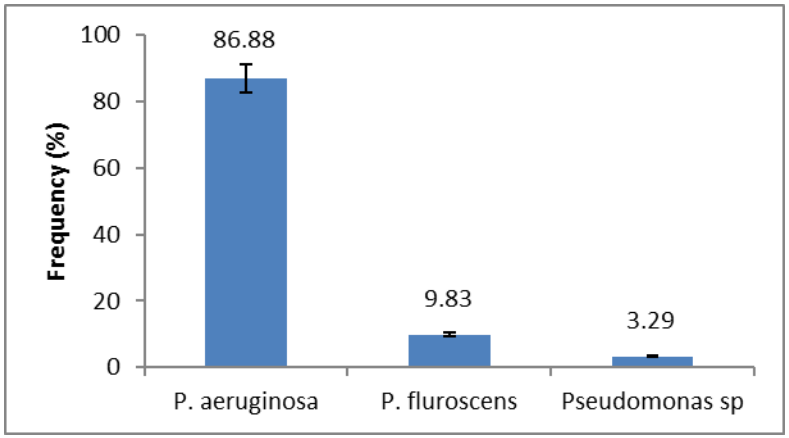

Figure I: Comparative prevalence of Species of Pseudomonas isolated.

Different species of *Pseudomonas* were isolated from the wounds of the patients, where *P. aeruginosa* ranked highest with Fifty- three 53(86.88%) followed by *P. fluorescens* 6(9.83%) and *Pseudomonas* sp. 2(3.29%), as shown in the figure above.

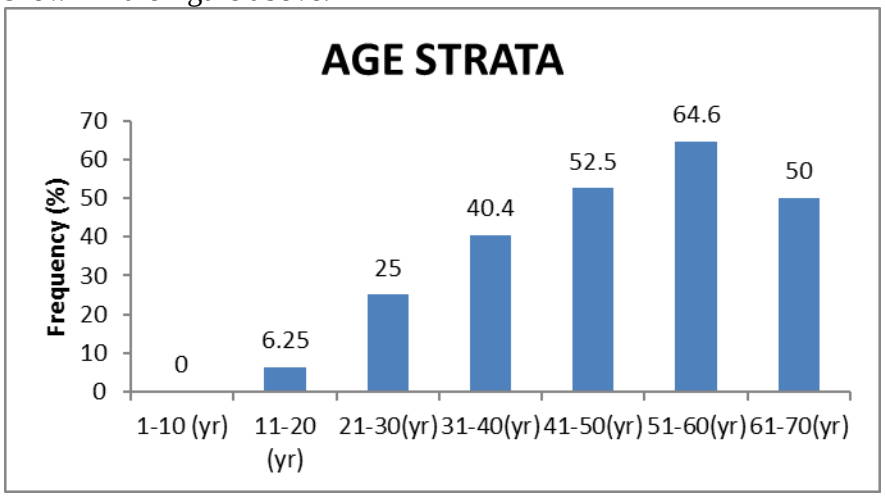

Figure II: Occurrence *P. aeruginosa* by age of the patients. Statiscally significant at (P<0.05)

The distribution of the bacteria among various age groups of the patients in the figure above indicated that there is association between the age of the patients and the occurrence of the bacteria in their wounds. This implies that age is statistically significant at (P< 0.05).

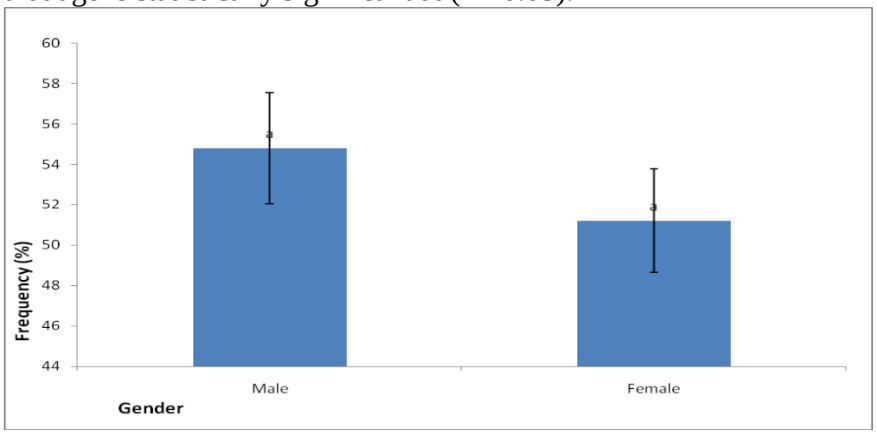

Figure III: Occurrence of *P. aeruginosa* by the gender of the patients.

Gender is statistically not significant (P>0.05).

Following the distribution of *Pseudomonas aeruginosa* among male and female patients, no statistical significance was shown (P>0.05) as indicated in the figure above.

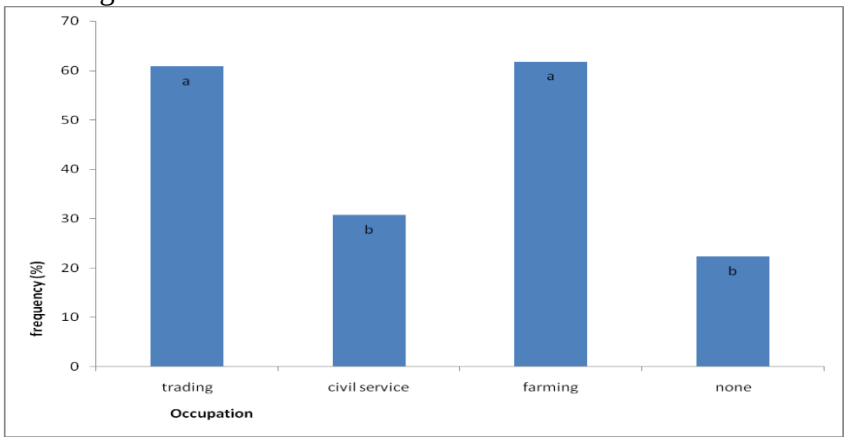

Figure IV: Occurrence of *P. aeruginosa* by the occupation of the patients.

Bars bearing different alphabets are statistically significant (P<0.05).
The distribution among different occupational groups indicated that the occupation of the patients is statistically significant judging by the significant difference (P<0.05), therefore, occupation is a determining factor for *Pseudomonas aeruginosa* to be present in a patient's wound.

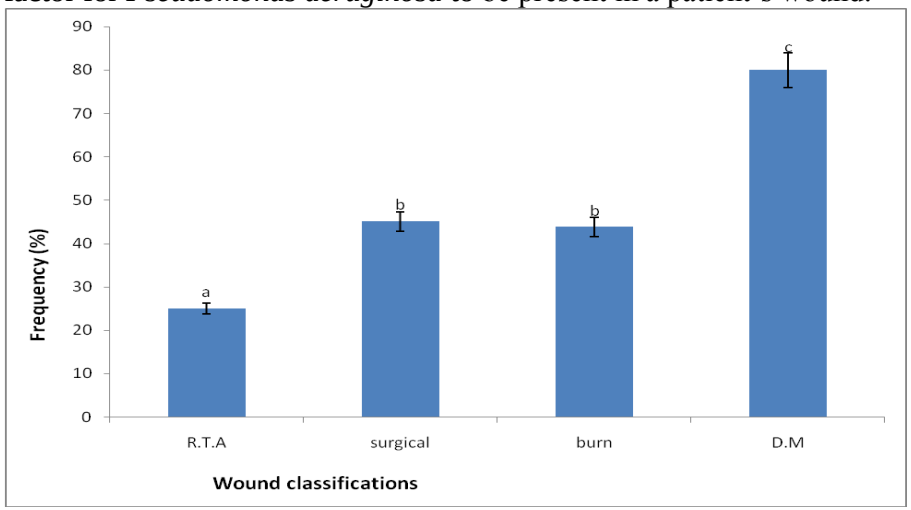

Figure V: Occurrence of *P. aeruginosa* by the cause of wound of patients.
Bars bearing different alphabets are statistically significant (P<0.05).
The different causes of wound is statistically significant (P<0.05), therefore the different causes of wound becomes significant to determine whether the wounds of the patients contain *Pseudomonas aeruginosa* or not.
KEY:
DM = Diabetes mellitus.
R.T.A= Road traffic accident

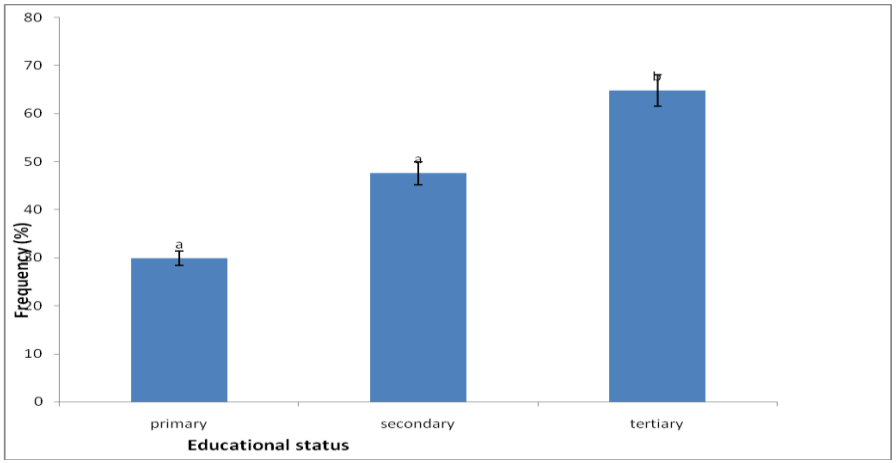

Figure VI: Occurrence of *P. aeruginosa* among patients of various educational backgrounds.
Bars bearing different alphabets of the Educational status are statistically significant (P<0.05) as indicated in the graph above.

TABLE I: Percentage Susceptibility of the Isolates to Antibiotics.

Antibiotics	Disc Potency(µg)	P. aeruginosa n = 53	P. fluorescens n = 6	Pseudomonas sp. n = 2
OFX	10	86.8 (13.2)	33.3 (66.7)	100 (0)
PEF	10	56.6 (13.4)	16.7 (83.3)	0 (100)
CPX	10	94.3 (5.7)	83.3 (16.7)	50 (50)
AU	30	73.5 (26.5)	50 (16.7)	50 (50)
CN	10	20 (37.7)	0 (100)	50 (50)
S	30	0 (100)	0 (100)	0 (100)
CEP	10	37.7 (62.3)	33.3 (66.7)	50 (50)
NA	30	28.3 (71.7)	16.1 (71.7)	50 (50)
SXT	30	3.7 (96.3)	0 (100)	0 (100)
PN	30	0 (100)	0 (100)	0 (100)

Susceptibility to antibiotics is shown outside the parenthesis, while resistance is shown within the parenthesis. Analyzing the Susceptibility result, *P.aeruginosa* isolates showed high Susceptibility to Ciprofloxacin, Tarivid and Augmentin, and were 100% resistant to Streptomycin and

Ampicillin. *P. fluorescens* isolates showed high Susceptibility to Ciprofloxacin only but were 100% resistant to Gentamycin, Streptomycin, Septrin and Ampicillin. *Pseudomonas* sp exhibited 100% Susceptibility to Tarivid and equally resisted Reflacin, Streptomycin, Septrin and Ampicillin by 100%.

KEY:

OFX = Tarivid. PEF = Reflacin. CPX = Ciprofloxacin. AU = Augmentin.
CN = Gentamycin. S = Streptomycin. CEP = Ceporex. NA = Nalidixic acid.
SXT = Septrin. PN = ampicillin.

DISCUSSION

This study implicated *Pseudomonas aeruginosa* and other species of the bacteria as possible etiological agents in the cases of wound infections observed. These bacteria includes; *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Pseudomonas* sp. Among the isolated bacteria, *P. aeruginosa* had the highest prevalence of 86.88%, and this is because it is a gram-negative Bacteria which is highly associated with wound infections, this is supported by the report of Kehinde *et al.* (2004) and Anupurba *et al.* (2010), but in contrast to the study carried out by Opalekunde *et al.* (2014). The predominant nature of the organism might be due to its presence in virtually all environments and even hospitals, possession of virulent properties which aids adherence to host tissues, which agrees with the study carried out by Opalekunde *et al.* (2014) which states that high prevalence of *P. aeruginosa* may be due to endogenous source of infection, and Cruciani *et al.* (1998) who reported that strains of *P. aeruginosa* is constantly being isolated from medical equipment and biofilm surfaces.

Demographic data of positive cases of infection due to the bacteria were examined by age strata and it was shown that there is a statistical significant difference ($P<0.05$), which agrees with a research report of Prvitts *et al.* (2001) which states that wound infection occurs more in adults than in children. Occupational factor was also assessed and it showed that occupation is a predetermining factor in the occurrence of wound infection due to *P. aeruginosa*, which means that it had statistical significance($P<0.05$). Occurrence of *P. aeruginosa* by the cause of wound was also analyzed and it showed a Statistical significance ($P<0.05$), and this goes to show that wound colonization by the bacteria may depend on the type of wound. The result shows that *P. aeruginosa* may not frequently colonize wounds due to road traffic accidents, because, they are fresh wounds, the Bacteria may be highly associated with surgical wounds due to the presence of the bacteria in hospital environments and surgical equipments. Prevalence in burn may be because, burn wounds are sometimes fresh and also because large area of the wounds are usually exposed, leading to high level of bacteria proliferation and lastly, highest prevalence was seen in wounds due to diabetes mellitus, because of difficulty in wound healing, the usual lengthy period of wound occurrence, which may cause the wounds to become infected during the process of treatment in the hospital and in other environments. This finding is supported by work done by Garba *et al.* (2014), where it was stated that exposed tissues in burn wounds are ideal for bacterial proliferation, and that *P. aeruginosa* is always isolated from surgical wounds. Educational status was also examined statistically and a significant difference was obtained ($P<0.05$). Statistical analysis of gender did not show any significance ($P>0.05$), this in contrast to the study carried out by Srinivas *et al.* (2001).

The susceptibility of *P. aeruginosa* isolates to antibiotics used differs. Out of the fifty-three (53) positive *P. aeruginosa* isolated, 46 (86.8%) were sensitive to Ofloxacin (tarivid) and Ciprofloxacin, 39 (73.5%) were sensitive to Augmentin, 30 (56.6%) were also sensitive to Reflacin and exhibited 100% resistance to Streptomycin and Ampicillin, which may be due to over use of this antibiotic, this study is supported by research findings of Garba *et al.* (2014) where it was stated that *P.* aeruginosa was susceptible to Ofloxacin and Augmentin, but in contrast, *P. aeruginosa* was sensitive to Ampicillin. This finding in this study is also in agreement with Opalekunde *et al.* (2014) which stated that *Pseudomonas* sp are resistant to Streptomycin and Ampicillin, it was also reported that *P. aeruginosa* was sensitive to Ciprofloxacin. The resistance shown to Streptomycin and Ampicillin may be due to usage over a long period of time, the inconsistency of some patients to the use of antibiotics and the rate in which they have been absorbed into the blood stream. This is in conformity with the findings of Mohammed *et al.* (2013), where it was stated that the over-use of antibiotics contributes to a patient developing resistance to a particular antibiotic. Sensitivity to Ofloxacin and Ciprofloxacin may be due to the fact that they are broad spectrum, less readily available to

patients, because they are rare and expensive, which means they may not have been miss-used as earlier reported by Vorland (2001), who stated that the success of Ciprofloxacin could be due to its broad spectrum activities which occurs in bacteria in both resting and replicative phases in order to destroy the deoxyribonucleic acid (DNA) and cause death of cell.

Conclusion and recommendations

Pseudomonas aeruginosa is the predominant organism isolated from wound samples screened in this study. This study was found to be in line with previous reports in which *P. aeruginosa* was found to be frequently isolated from infected wounds. The high resistance shown to some antibiotics can be due to inappropriate use of drugs. There is therefore a need to put in place effective surveillance measures in a bid to check the spread of infection due to the studied organism. *Pseudomonas aeruginosa* was discovered to be the most important cause of morbidity and mortality in patients whose wounds are infected. This study highly recommends the use of Ofloxacin, Ciprofloxacin and Augmentin for treatments of wound infections due to the study Bacteria.Efforts should be made by the government of the country and states to create awareness on hygiene practices and on proper use of antibiotics with emphasis on the inherent danger of self medication. Caregivers should make efforts to stay up to date on the susceptibility pattern of *P. aeruginosa* and other organisms predominant in wound infection and other cases of infection. Patients should be sensitized on the need to report wounds early enough to the Hospital to avoid wound degeneration.

Acknowledgement

We are indeed grateful to the management of the General Hospital, Minna, for giving us the opportunity to carry out this research in the hospital. We also appreciated the co-operation from the Doctors, Nurses, and the entire staff of the Laboratory for allowing us to have access to the consented patients, and thereby contributing immensely to the overall success of this research.

Conflict of Interest.

No conflict of interest.

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Effect of Fungal Infection on the Anatomical Integrity of Cells of Some Selected Tubers (*Colocasia esculenta*, *Dioscorea alata* and *Ipomoea batatas*)

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Abstract

Tubers are staple food crops in most parts of West Africa as they serve as a major source of energy to the consumers. The fungi associated with *Colocasia esculenta*, *Ipomoea batatas* and *Dioscorea alata* in storage were isolated on PDA plates using the pour plate technique. The effects of the fungal hyphae on the anatomical features of the three tubers were also studied using light microscopy. *Trichoderma harizanum, Rhizopus stolonifer, Mucor racemosa, Aspergillus niger, Aspergillus flavus, Aspergillus brasiliensis, Aspergillus fumigatus, Botrydioplodia theobromae, Penicillium chrysogenum, Ceratocystis fimbriata, Trichoderma viridae, Aspergillus terrus, Fusarium oxysporium* and *Aspergillus uvarum* were identified as fungi associated with the tuber spoilage. Anatomically, the intercellular and intracellular walls of the tissues were greatly disrupted. The structural integrity of the parenchyma cells were negatively affected though the level of hyphae penetration is superficial. Indeed, the relatively harder parts- inner cortex parenchyma area and core amyliferous layer were not yet affected by the hyphal growth most probably because the period of infection was early. This study showed that *Aspergillus* species were the most isolated fungi and also inferred that though destruction of food crops may not be visible during storage, consumption of infected tubers can lead to adverse health effect which can be avoided.

Key words: Fungal infection, tubers, pathogenic fungi, tuberous tissue, parenchyma cells.

INTRODUCTION

Postharvest diseases caused by fungi constitute a worldwide public health problem and preventing them is a major goal of societies. Microbiological foodborne diseases are typically caused by fungi or their metabolites, parasites, virus or toxins (Oloruntoba, 2015). Some fungi produce poisons in food without signs of spoilage and consumers may therefore be unaware of the contamination. Tuber crops are moist, low acidic foods that can support the growth of food poisoning fungi. Tubers are staples found in many parts of the tropic regions of the world and they belong to the class of food that basically provides energy in the human diet. There are several stem tubers (FAOSTAT 2015). The main components of tubers are water (60-90%), starch and fiber, with lesser amounts of proteins, fat, sugars, vitamins and minerals (FAO, 2006). Tubers are cheap but nutritionally rich staple foods which are associated with high transportation cost, short shelf life, and limited market margin in developing countries (Chandrasekara and Kumar, 2016).

Ipomoea batatas L (Lam) the seventh largest food crop, grown in tropical, subtropical, and warm temperate regions in the world (Agu *et al.*, 2015) is an important food crop in Nigeria ranking third amongst important tuber crops of Sub-saharan Africa, after yam (Anoma and Joseph, 2016) while the vines are fed to livestock. Quantitative and qualitative losses of sweet potato arising from post-harvest storage results from physical, physiological, or pathological factors or various combinations of these factors (Ogbo and Agu, 2014). *Dioscorea alata* (water yam) is ranked next to *Dioscorea rotundata* (white yam) (Pandey, 2007). It is a staple food crop as well as a cash crop in West Africa, especially in Nigeria.

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Africa is the largest producer of yams in the world where it is a staple food as well as a means of foreign exchange (Pandey, 2007). In some countries where there is no white yam, *D. alata* production is the highest amongst yams. Unlike the white yam which is perennial *D. alata* is known as ten months' yam because it takes nine to ten months to mature (Onuh *et al.*, 2015). The high phytochemicals (phytate, lectins, phenolic compounds, amylase inhibitors and saponins) in *D. alata* are believed to be of great disease treatment value; these are reported to reduce blood glucose, plasma cholesterol, triglycerides level and control cancer risks (Onuh *et al.*, 2015) and often recommended for diabetic patients and those slimming due to its low glycemic index. *Colocasia esculenta* (cocoyam), otherwise known as 'koko' among the Yoruba tribe of Nigeria has the ability to withstand waterlogged and reduced conditions (Awah *et al.*, 2016), since it can transport oxygen from the leaves to the roots under such conditions. *C. esculenta* grows well on all types of soils, the ideal type being deep well drained, friable loams, particularly alluvial (Pandey, 2007). Cocoyam is used as source of food for man and livestock (Agu *et al.*, 2016). Therefore, this study aimed at isolating and identifying various fungal species associated with sweet potato, water yam and cocoyam spoilage with a view of highlighting the effect of the fungal infections on the anatomical features of the tubers.

MATERIALS AND METHODS

Source of Microorganisms

Samples of fresh and infected tubers of *Colocasia esculenta*, *Ipomoea batatas* and *Dioscorea alata* were purchased between the months of May and July, 2017 from three localities (Egbeda market, Ayobo market and Bariga market) in Lagos, Nigeria. These were singly placed in sterile labelled polythene bags and transported to the lab. The infected tubers showed some rot symptoms of black spots, browning, pale colours and unpleasant odour. While the fresh tuber samples were still in their natural fresh colours without blemish which showed that they were still healthy. After the collection of these samples from study sites, they were then carried to the Botany Research Lab, University of Lagos where fungal pathogens were isolated from the infected tubers.

Isolation of Fungi from Samples

The isolation technique of Samuel and Adekunle (2015) was adopted in this study. A small section of infected *C. esculenta*, *I. batatas* and *D. alata* tissues containing the advancing margin of rot and adjoining healthy tissue were cut using sterilized scalpel and cork borer while the surfaces were sterilized by dipping completely in a concentration of 40% hypochlorite solution for 60 seconds; the sterilized sections to be inoculated were then removed and rinsed with three changes of sterile distilled water. The tuber pieces were made to dry by blotting with sterile filter paper in a laminar airflow cabinet. With the aid of a sterile forceps four pieces of each cut samples were separately inoculated (90° apart) on solidified potato dextrose agar (PDA) plates which were prepared according to manufacturer's specification (Oxoid, Basingstoke, England). Two replicates for each sample were made. The plates were incubated a temperature of 28-30 °C in an incubator. After 72 hours, emerging fungal colonies were sub cultured individually on new PDA plates until pure cultures were obtained. Fungi associated with the tubers spoilage were observed.

Identification of fungi

For identification, morphological studies of the pure culture plates, i.e., the shape, size and spore formation after 72 hours were observed. The mycelium containing the spores were teased out on a slide with the aid of an inoculating needle cleaned with ethanol and subsequently stained with lactophenol blue and was observed under light microscope. Microscopic features of the organisms were taken using Motic Camera 2000. The fungi identified were confirmed by comparing their morphology with fungi descriptions of Talbot (1971), Deacon (1980) and Bryce (1992).

Anatomical analysis

Thin sections of the tissues were obtained from five different layers of both diseased and healthy tubers namely: secondary tissue, cambial layer, sub-apical meristem, primary tissue and delayed multiplicating layer (Fig. 1) (Queva, 1894; Degras and Mathurin, 1978) for the study. Sectioning was done with the aid of sharp dissecting blade because of softness of the tissue. Several thin slices of the tissues of *C. esculenta*, *I. batatas* and *D. alata* used for the study were obtained from longitudinal, transverse, radial and tangential sections. Cell contents were eliminated using Sodium hypochlorite (Commercial bleach) while the cells were hardened with some drops of 50%-100% ethyl alcohol that were added in series. Each preparation was stained with Safranin O, the mountant used was glycerin and then, they were covered with cover slips and the edges of the slips were coated with nail polish. The features of each sample were captured using a microscope camera

'Toup view 3.2" attached to an Olympus microscope.

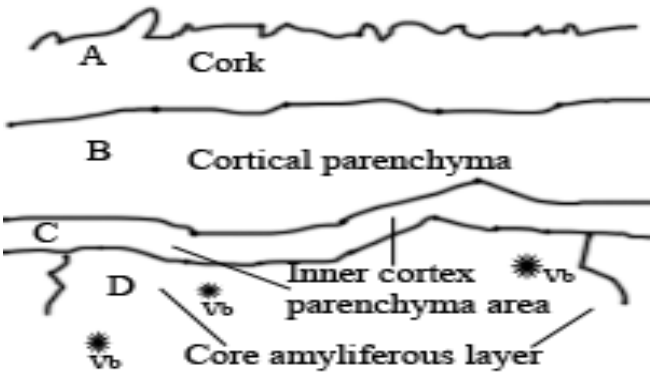


Fig. 1 A schematic diagram of layers of cells of tuberous tissue. Fahn (1967).

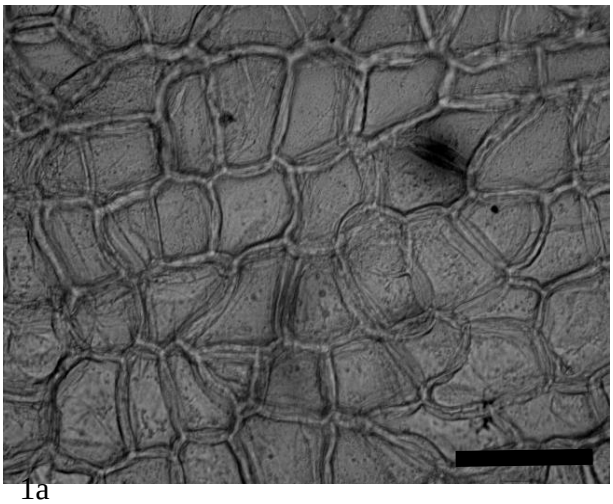
RESULTS

Identification of Fungi Isolates

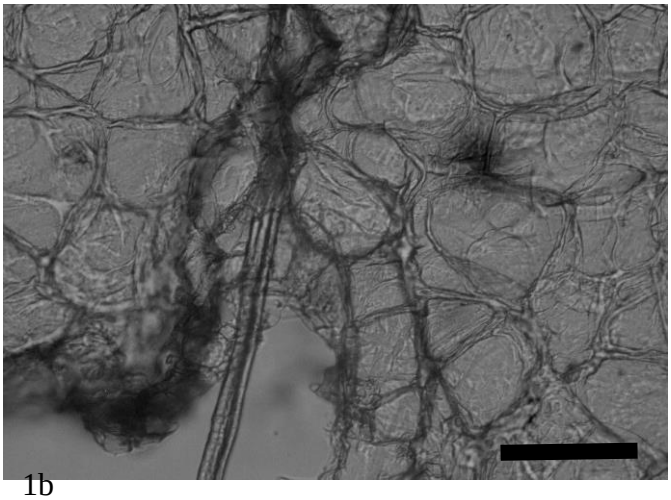
A total of fifteen different kinds of fungi were isolated on the PDA medium from infected water yam, cocoyam and sweet potato. These fungi isolates are; *Trichoderma harizanum, Rhizopus stolonifer, Mucor racemosa, Aspergillus niger, Aspergillus flavus, Aspergillus brasiliensis, Aspergillus fumigatus, Botrydioplodia theobromae, Penicillium chrysogenum, Ceratocystis fimbriata, Trichoderma viridae, Aspergillus terrus, Fusarium oxysporium* and *Aspergillus uvarum.*

Anatomical review on epidermal layers of selected tubers

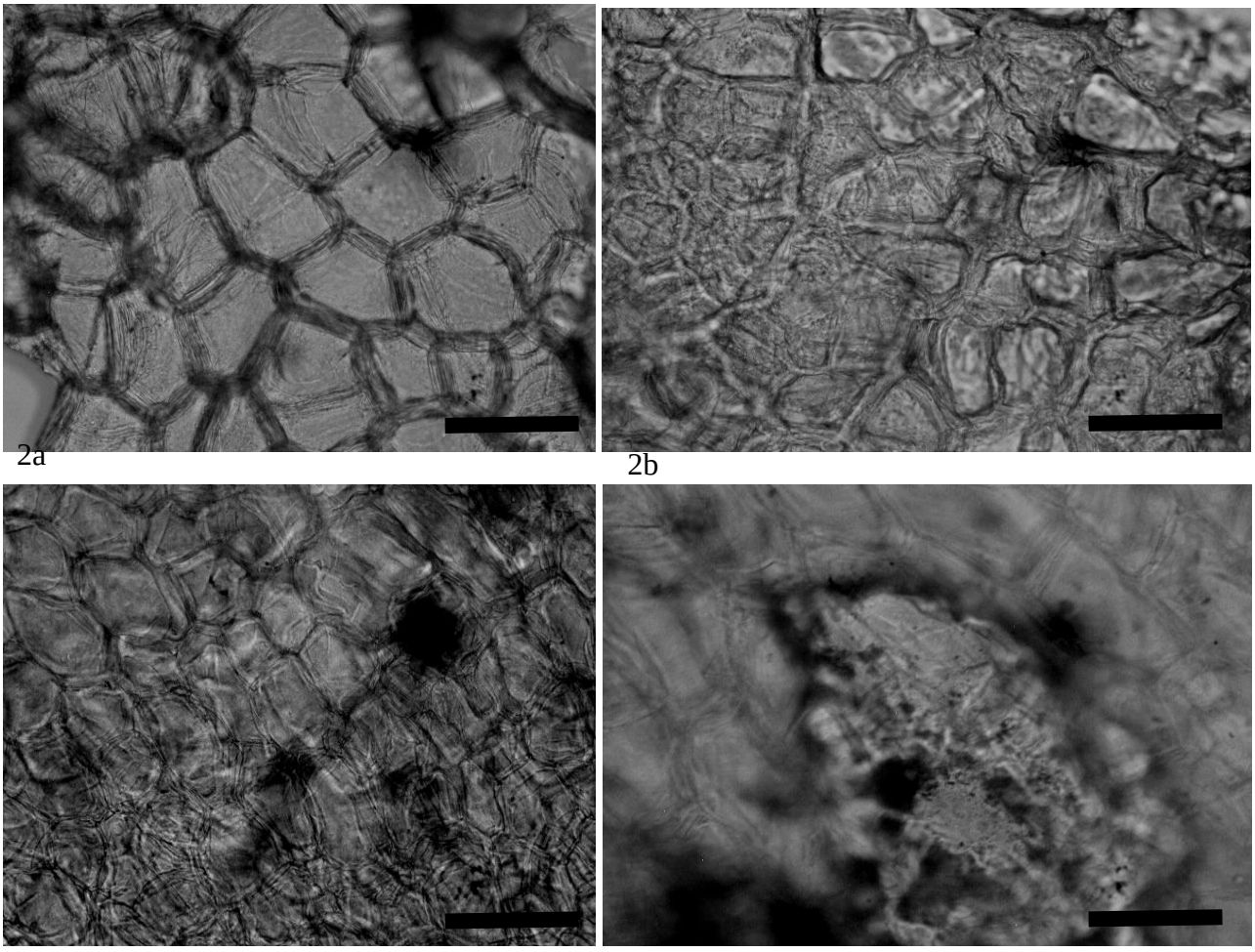
The five different layers of both diseased and healthy tubers namely: secondary tissue, cambial layer, sub-apical meristem, primary tissue and delayed multiplicating layer revealed different features of the samples. An illustration of these layers is presented in Figure 1. The sign of infection was noticeable in the secondary tissue, a layer that is next to the cortical layer. Infection is still partially superficial in the tissues examined, hence it has not penetrated deep to the amyliferous zone where there is higher food reserve. Infections were visible in all sections; but more features of the tissues were seen through the longitudinal section. The affected cells are parenchymatous (Fig. 1). In the sweet potato tubers (*Ipomoea batatas*), the healthy tissue, parenchyma cells have normal configuration (Plate 1a) whereas, symptoms of spoilage were indicated by ruptured tissues and there was an evidence of growing hyphae in the tissue (Plate 1b). In the spoilt water yam, parenchyma cells appeared broken and roughened and the cell walls were damaged (Plate 3a, b). For the cocoyam, the cell walls and contents were distorted and there was growth of fungal hyphae penetrating the cells (Plate 2b).



1a



1b



2a 2b

3a 3b

Plate…..Photomicrographs of the parenchyma cells of the tubers of *Ipomoea batatas*- Plate 1a: Fresh (Mag x400), Plate 1b: Infected (Mag x400); *Colocasia esculenta*- Plate 2a: Fresh cocoyam (Mag x400). Plate 2b: Infected cocoyam (Mag x400) and *Dioscorea alata*- Plate 3a: Fresh (Mag x400), Plate 3b: Infected (Mag x400). Scale bar 25µm.

DISCUSSION

Tuber crops which are economical to man due to the way they are handled from the field during cultivation, harvesting, packaging and transportation before finally marketing them are mostly exposed to pathogenic fungi. Most of these pathogenic fungi may not reflect their symptoms at the early stages and could cause harmful infections to liable consumers. Tubers that develop symptoms early enough are separated from the healthy ones which result in economic loss to farmers and marketers. Most of the fungi we isolated from this study have been reported to cause deterioration and losses to agricultural products from so many parts of the world (Oyeyipo, 2012; Ogbo and Agu, 2014; Onuh *et al.*, 2015). Observation during this study showed that fungi grew easily with available nutrients (PDA) which affirms that once there is sufficient nutrient supply fungi flourish thereby affecting the healthy condition of the host in this case the agricultural produce. Members of the genus *Aspergillus* were the most frequent observed in our study supporting other findings that this group of fungi causes deterioration in farm produces like tubers during storage. The results from this study correlates with the reports of Essono *et al.* (2007), Onuh *et al.*, (2015) and Ogunleye *et al.* (2014). These authors reported *Aspergillus niger* as an acute agent of tuber decay.

The isolation and identification of *Rhizopus* and *Mucor* species from this present work also aligned with the report of Agu *et al*. (2016) that these two fungi belong to

the fast growing group of fungi which cause rot in cocoyam.

The study also surmised the presence of *Botrydioploda theobromae* in the diseased water yam. It is remarkable to note that *B. theobromae* sporulates on several varieties of *Dioscorea* media because the fungus has curiously been found associated with the storage of yams (Nwawuisi *et al.*, 2012 and Adeogun *et al.*, 2014). Furthermore, *B. theobromae* identified in this study has been reported to cross infect other crops like cocoa, mango, banana and yam with significant tissue damage and economic losses.

The intercellular and intracellular connection of the penetrating fungal hyphae greatly disrupted the structural integrity of the parenchyma cells of the studied tubers. Anatomy of the sweet potato, cocoyam and water yam observed. This was very obvious in the sweet potato samples where hyphae were spotted causing lesions in the parenchyma cells and the water yam where the infection was in the advanced stage which led to destruction of the cell properties. The cortical parenchyma of the three studied tubers was affected by the fungi. Fungal hyphae were noticeable in the affected tissues of *Ipomoea batatas* and *Colocasia esculenta* whereas, total rupturing of the tissue was observed in the damaged tissue of *Dioscorea alata*. It is evident that the level of infection is still superficial in all the three species. Hyphal growth has not penetrated to the inner cortex parenchyma area and core amyliferous layer. Ayensu (1972) reported that striking uniformity of the general anatomy can be observed in the tubers of Dioscoreaceae. The inner cortex and the amyliferous parenchymatous layers can be lignified and possess some strengthening substances (Mantell *et al*. 1977). This may hinder the growth of fungal hyphae in the tissue as the tissues were macerated. Similarly, Davies *et al*., (1981) noted an extensive maceration of colonized tissues of carrot root in storage by *Mycocentrospora acerina*. They reported that the obstruction imposed by the network of invading hyphae impaired the proper functioning of the tuber tissues which may result into rot. According to Impullitti and Malvick (2014), penetration of fungal hyphal can cause a disruption in vessel functions which also leads to disruption in water movement. They also stated that colonization of pathogenic fungi hyphae may leads to abnormal function of the tuber tissues thereby resulting into rot and anatomical changes as observed in the tubers.

Our report has also shown that though some tubers may appear physically healthy to consumers, they could pose a threat to consumers' health. Fungi pathogens though are microscopic in nature, cause damages and deterioration before the symptoms become visible. The difference can be seen on the anatomy between the healthy and infected tubers where invasion and colonization of host tissues is noticed in some tubers until the whole cell is degraded.

CONCLUSION

Postharvest spoilage by pathogenic fungi has been found to reduce economic shelf life in tuber crops. For there to be an increase in economic value and safety of lives, postharvest handling and facilities should be improved to avoid losses and meet the economic demand of tuber crops in Nigeria. Proper practices and modern technologies should also be employed for better storage and preservations.

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CONFLICT OF INTEREST

Authors declare that no conflict of interest.

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Detection and Antibiotic-resistance of *Salmonella* species and *Escherichia coli* from Selected Captive Animals in Ogba Zoological Garden

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ABSTRACT

Animals kept at the zoo are customarily raised in confinement, captured in the wild or acquired from other facilities. They have been reported to be connected with *Escherichia coli* and *Salmonella* infections with a significant hazard to public health. This study was designed to elucidate antimicrobial susceptibility profile of *Salmonella* species and *Escherichia coli* isolated from captive animals in Ogba Zoological Garden. A total of 60 faecal samples were randomly collected from captive animals between April and June 2017 and analyzed using standard culture-based techniques. Antimicrobial susceptibility profile of the isolates was carried out using the disk diffusion method. The mean cell density was $4.0\times10^{8}\pm0.60$ cfu/g for heterotrophic bacteria; $7.9\times10^{5}\pm1.28$ cfu/g for *Escherichia coli* and $1.0\times10^{6}\pm2.81$ cfu/g for *Salmonella* species. All faecal samples examined in this study were positive for *Salmonella* species and *E. coli*. A total of 38 *E. coli* and 44 *Salmonella* species were selected and subjected to antibiogram characterization. Antibiogram findings revealed that 38/38 (100%) of the *E. coli* isolates were resistant to 3 antibiotics (cefepime, meropenem, tetracycline) which belongs to 3 antimicrobial class (cephems, carbapenems and tetracyclines) with multiple antibiotic resistant index of 0.25; while 44/44 (100.0%) of the *Salmonella* isolates were resistant to 3 antibiotics (cefepime, ertapenem, tetracycline) which belongs to 3 antimicrobial class (cephems, carbapenems and tetracyclines) with multiple antibiotic resistant index of 0.27. However, 36/38 (94.7%) and 30/38 (78.9%) of the *E. coli* isolates were sensitive to cefotaxime and ceftriaxone respectively. Also, 12/44 (27.3%) and 14/44 (31.8%) of the *Salmonella* isolates were sensitive to ceftriaxone and gentamycin respectively. The presence of these potential pathogens in the faeces of captive animals coupled with their multidrug resistance potential call for public health concern as this could be a route of transfer of multidrug-resistant pathogens in the environment.

Keywords: Captive animals, Zoo, *Salmonella*, *Escherichia coli*, Multidrug-resistant, Public health

Introduction

Interactions with animals afford numerous benefits to adults and children via entertainment and education. Many health benefits of the animal-human interface have been acknowledged and comprise lowered blood pressure and reduced anxiety (Dunn *et al*., 2015). Contact with catchment animals occurs in a variety of public settings, such as petting zoos, rodeos, farm tours or visits, livestock exhibitions, and state or county agricultural fairs (Pickering *et al*., 2008; Conrad *et al*., 2017). Despite the numerous benefits of public catchment centres, there are significant risks involved if proper hygiene measures are not practised. Of significant importance are zoonoses (or zoonotic diseases), which are infections that can be disseminated amongst humans and animals.

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These disease-causing agents may be disseminated in animal faeces and disseminated to humans via faecal-oral routes and the environment. The study of disease-causing microorganisms from animal populations kept in the Zoo is crucial for execution of programs for prevention and control as well as surveillance of infectious diseases, for the advancement of animal and public health policies.

Studies have shown that infants and children less than 5 years are at the apex risk of obtaining zoonotic pathogens from animals (Dunn *et al*., 2015). Often times, this is ascribed to attraction to or inquisitiveness about animals, poor hygienic practices, and an immune-compromised or inadequate immune system (Pickering *et al*., 2008; Dunn *et al*., 2015). Often times, the symptoms and clinical expressions have been recounted to be more morbid in young children and infants (Pickering *et al*., 2008). In some scenarios, the zoonotic illnesses are avoidable via improved hygiene (Erdozain *et al*., 2015; Conrad *et al*., 2017). However, the symptomatology and consequences of such expressions have been reported. Individuals irrespective of their age with secondary or primary immune-deficiency are exposed to even more severe diseases, with the elderly and antenatal women not excluded (Pickering *et al*., 2008). Most often, zoonotic infections are microbial complications which emanate from the gastrointestinal tract, with morbid to fatal outcomes ranging from abdominal cramps, vomiting and diarrhoea to bloody diarrhoea, kidney failure, and, in severe scenarios, death (Dunn *et al*., 2015).

Salmonella species have been described to produce asymptomatic as well as symptomatic clinical manifestations in humans and animals, with symptoms which include fever, diarrhoea, abortion, vomiting, osteomyelitis and intermittently death (Stevens *et al*., 2009). Also, *Escherichia coli* are commensal bacteria that are usually non-pathogenic and reside within the large intestine of warm-blooded animals and human (Beshiru *et al*., 2016; Marchant *et al*., 2016). Additionally, some strains of *E. coli* and *Salmonella* have been reported as a global zoonotic pathogen that can result in bloody diarrhoea, haemolytic uremic syndrome and hemorrhagic colitis in humans (Chaudhuri and Henderson, 2012). *Salmonella* species have been identified from captive animals with asymptomatic manifestations (Stirling *et al*., 2008; Jardine *et al*., 2011), and has also resulted in disease outbreaks with increased death rates (Marchant *et al*., 2016). In the case of *E. coli*, reports in captive animals have revealed variable prevalence in the range of 0.1% to 50.8%, probably as a result of the different detection/isolation procedures and species of animals being studied, though asymptomatic manifestation is always described (Stirling *et al*., 2008).

Antibiotic-resistant bacteria are significantly important to human health, but the reservoirs of resistance determinants are poorly understood. The genesis of antimicrobial resistance in catchment animals is significant to human health due to the cumulative importance of zoonotic diseases and the necessity for predicting the development of resistant pathogens (Radhouani *et al*., 2014). Proximity to human activities impacts the antibiotic resistance profiles of bacteria in captive mammals, which reside in densely populated microbial habitats where antibiotics select for resistance. Though numerous bacterial species are significant in terms of multidrug resistance in veterinary and human medicine, *E. coli* and *Salmonella* are considered as key bacterial pathogens to monitor the advancement of multiple drug-resistant bacteria in captive animals (Radhouani *et al*., 2014).

Salmonella cases emanating from humans have been tied to direct or indirect interaction with animal sources in several independent reports (Hernandez *et al*., 2012; Hauser *et al*., 2012; Lawson *et al*., 2014). The majority of those reports relied chiefly on post-outbreak surveys with few utilizing molecular typing to investigate similarities between human and animal isolates (Hernandez *et al*., 2012). Both *E. coli* and/or *Salmonella* species have been detected from several captive animal species in Chile (Marchant *et al*., 2016), Malaysia (Saleha *et al*., 2015), USA (Hernandez *et al*., 2016), Brazil (Suphoronski *et al*., 2015), Iran (Koochakzadeh *et al*., 2015), Grenada (Amadi *et al*., 2015), Spain (Núñez-Díaz *et al*., 2017), Japan (Kabeya *et al*., 2017), Egypt (Mahmoud, 2015), and Sri Lanka (Kulasooriya *et al*., 2016) with limited information from Nigeria. This study was carried out to elucidate the antimicrobial susceptibility profiles of *Salmonella* species and *E. coli* in captive animals from Ogba zoological Garden, Benin City, Nigeria.

MATERIALS AND METHODS

Study Area

Ogba Zoo was established in 1965 in a forest reserve in Ogba District, four km from the city centre. Ogba Zoo (also known as Ogba Zoo and Nature Park) is an extensive state-owned multi-purpose recreational centre in 750 Acres of land as well as Urban Park with tourist resort development and Biological Garden. It is located between Oko and Ogba district with coordinate's 6°17'20" N 5°35'16" E; 5 km Airport road, Benin City, Nigeria. The Ogba Zoo is home to a number of local and wild species of animals including lions, primates, rock pythons, giant tortoise, antelope and equine species to mention a few. The Zoo opens at 9 am and closes at 6 pm. In addition, the Ogba Zoo and Nature

Park also have an exclusive event ground with open access and robust facilities that accommodates an average of 1, 500 persons with free space for side activities.

Sample Collection

A total of 60 faecal specimens were randomly collected from captive animals (Guinea fowl, Chimpanzee, Spotted hyena, Red Potas and Baboon) between April and June 2017. Faecal samples were collected from different points at the catchment center with the aid of sterile glass container, labeled appropriately and transported to the Applied Microbial Processes and Environmental Health Research Group Laboratory at the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City in cold ice pack and analyzed within 4 h after collection.

Isolation and identification of *E. coli* and *Salmonella* species

Samples were serially diluted and spread platted into separate Nutrient agar (NA) (Lab M, UK) for heterotrophic bacteria enumeration; Hektoen Enteric agar (HEA) (Lab M, UK) for *Salmonella* and Chromocult Coliform agar (CCA) (Merck, Germany) for *E. coli* and incubated at a 37±2º C for 18-24 h. After incubation, discrete colonies were counted and expressed in colony forming unit per gram. The colour of *Salmonella* sp. on the HEA agar plate was observed as green with dark centres as well as completely black isolates, while the colour of *E. coli* on CCA was observed as purple to violet colour for presumptive identification and enumeration. Enrichment using Selenite F-Broth for *Salmonella* species (Lab M, UK) and Tryptone Soy Broth (Merck, Germany) for *E. coli* was incubated for 18-24 h at 37 º C. Thereafter, streak plate technique was employed to streak directly on HEA and CCA plate and incubated for another 18-24 h at 37ºC (Igbinosa, 2015). Distinct colonies were repeatedly sub-cultured, purified on nutrient agar and stored at refrigeration temperature for further analysis. Gram reaction with 3% KOH test; oxidase and catalase assessments were carried out. Gram-negative, catalase-positive and oxidase negative isolates were suspended in normal saline (0.8%) and turbidity adjusted to 0.5 McFarland standard. The bacteria suspension was inoculated onto API 20E strips (bioMérieux, Marcy-L'Étoile, France) following user' manual, strips were incubated for 24 h and read. The results were interpreted using API 20E software).

Antibiotic Susceptibility Test

The *Salmonella* and *E. coli* isolates that were positively identified using the culture-based method were subjected to antibiogram characterization. All the bacterial isolates were assessed for sensitivity or resistance to standard antibiotics using the Kirby-Bauer test method. For the disc diffusion assay, bacteria were grown between 18 and 24 h on Mueller-Hinton agar, harvested and then suspended in 0.85 % saline solution adjusted to a 0.5 McFarland turbidity standard. The inoculum was streaked onto plates of Mueller-Hinton agar (Merck, Germany) using a sterile cotton swab and impregnated with appropriate antibiotics. The outcomes were documented after incubating for 24 h at 37ºC. Commercially available antibiotics disc (Mast Diagnostics, Merseyside, United Kingdom) were used to elucidate the resistance forms of the isolates against ten different antibiotics (1 dose/disc) grouped into seven different classes of antibiotics. The width zone of inhibition surrounding each disc was measured and interpreted as resistance (R), intermediate (I) or sensitive (S) in accordance with the recommended standard established by the Clinical Laboratory Standard Institute (2017).

Statistical Analysis

All data were statistically analyzed using the statistical package IBM SPSS Statistics, version 21.0. All values in triplicates were analyzed using descriptive statistics and expressed as the mean ± standard deviation of the mean.

RESULTS

The population density of the bacterial isolates

The mean population density of the bacteria isolated from the faeces of the captive animals is presented in Table 1 below. The total heterotrophic bacteria ranged from 3.0×10^7 to $4.5 \times \text{cfu}10^{10}$ cfu/g with a mean cell density of $4.0 \times 10^8 \pm 0.60$ cfu/g. The *Escherichia coli* count ranged from 6.4×10^3 to 3.5×10^6 cfu/g with a mean of $7.9 \times 10^5 \pm 1.28$ cfu/g. The *Salmonella* species count ranged from 2.0×10^2 to 8.0×10^6 cfu/g with a mean of $1.0 \times 10^6 \pm 2.81$ cfu/g. A significant difference was observed in the population densities expressed ($p<0.05$). Findings from the selected captive animals studied [Guinea fowl (14), Chimpanzee (10), Spotted hyena (13), Red potas (13) and Baboon (10)] revealed that all samples harboured *Salmonella* species and *E. coli* respectively culminating in 100% prevalence.

Identification of *Salmonella* and *Escherichia coli* isolates from captive animals

The sum of 44 *Salmonella* and 28 *E. coli* were recovered from the faecal samples of captive animals. The identification was established on the basis of

biochemical characterization. The distribution of the *Salmonella* and *Escherichia coli* isolates in the different captive animals is as shown in Table 2.

Antimicrobial susceptibility profile of the bacterial isolates

The resistant profile of 38 selected and purified *E. coli* against 12 antibiotics belonging to 7 groups of antimicrobials (Table 3) revealed that 38/38 (100%) were resistant to cefepime (Cephems), meropenem (Carbapenems), and tetracycline (Tetracyclines). In addition, 36/38 (94.7%) were resistant to cefuroxime (Cephems), 32/38 (84.2%) were resistant to ceftazidime (Cephems), 22/38 (57.9%) were resistant to gentamicin (Aminoglycosides), with 20/38 (52.6%) resistant to aztreonam (Monobactam). Likewise, 36/38 (94.7%) were sensitive to cefotaxime (Cephems), 30/38 (78.9%) were sensitive to ceftriaxone (Cephems), 24/38 (63.1%) were sensitive to ciprofloxacin (Fluoroquinolone), while 20/38 (52.6%) were sensitive to piperacillin (Penicillins).

The resistant profile of 44 selected and purified *Salmonella* species (Table 3) on 11 antibiotics belonging to 7 groups of antimicrobials revealed that 44/44 (100%) were resistant to cefepime (Cephems), ertapenem (Carbapenems), and tetracycline (Tetracyclines). Furthermore, 42/44 (95.5%) were resistant to cefotaxime (Cephems), 34/44 (77.3%) were resistant to aztreonam (Monobactam), 32/44 (72.7%) were resistant to imipenem (Carbapenems), while 28/44 (63.6%) were resistant to ciprofloxacin (Fluoroquinolone). Contrarily, 14/44 (31.8%) were sensitive to gentamicin (Aminoglycosides), and 12/44 (27.3) to ceftriaxone (Cephems).

Multidrug-resistant profile of the bacterial isolates

Multidrug-resistant profile revealed that 3/38 (7.89%) of the *E. coli* isolates were resistant to 9 antibiotics (CPM^R, CAZ^R, CXM^R, ATM^R, MEM^R, IMI^R, GEN^R, TET^R, CIP^R) which belongs to 6 antimicrobial class with multiple antibiotic resistant index of 0.75. More so, 38/38 (100%) were resistant to 3 antibiotics (CPM^R, MEM^R, TET^R) which belongs to 3 antimicrobial class with multiple antibiotic resistant index of 0.25 (Table 4a).

Similarly, the multidrug-resistant profile of the *Salmonella* isolates revealed that 9/44 (20.46%) were resistant to 10 antibiotics (CTX^R, CPM^R, CTR^R, ATM^R, PIP^R, IMI^R, ETP^R, GEN^R, TET^R, CIP^R) which belongs to 7 antimicrobial class with multiple antibiotic resistant index of 0.91. More so, 44/44 (100.0%) were resistant to 3 antibiotics (CPM^R, ETP^R, TET^R) which belongs to 3 antimicrobial class with multiple antibiotic resistant index of 0.27 (Table 4b).

Table 1. The population density of the bacterial isolates

Population density	Minimum cfu/g	Maximum cfu/g	Mean ± SD cfu/g
Heterotrophic bacteria	3.0×10^7	4.5×10^{10}	$4.0 \times 10^8 \pm 0.60$
Escherichia coli	6.4×10^3	3.5×10^6	$7.9 \times 10^5 \pm 1.28$
Salmonella species	2.0×10^2	8.0×10^6	$1.0 \times 10^6 \pm 2.81$
p-value			0.001

Legend: Values presented in the table represent the mean ± standard deviation from the population density the entire samples.

Table 2. Distribution of *Salmonella* species and *E. coli* in faecal samples of captive animals

Captive animals	*Salmonella* sp.	*E. coli*
Guinea fowl	18 (40.9%)	13 (46.42%)
Chimpanzee	5 (11.3%)	7 (25%)
Spotted hyena	3 (6.8%)	5 (17.9%)
Red potas	7 (15.93)	4 (14.2%)
Baboon	11 (25%)	9 (32%)

Table 3. Antimicrobial susceptibility profile of *Salmonella* species and *Escherichia coli* recovered from fecal specimen

Antimicrobial class	Antibiotics	*Escherichia coli n*=38			*Salmonella* species *n*=44		
		R	I	S	R	I	S
Cephems	Cefotaxime 30µg	0 (0)	2 (5.3)	36 (94.7)	42 (95.5)	2 (4.5)	0 (0)
	Cefepime 30µg	38 (100)	0 (0)	0 (0)	44 (100)	0 (0)	0 (0)
	Ceftriaxone 30µg	0 (0)	8 (21.1)	30 (78.9)	10 (22.7)	22 (50)	12 (27.3)
	Ceftazidime 30µg	32 (84.2)	6 (15.7)	0 (0)	ND	ND	ND
	Cefuroxime 30µg	36 (94.7)	2 (5.3)	0 (0)	ND	ND	ND
Monobactam	Aztreonam 30µg	20 (52.6)	10 (26.3)	8 (21.1)	34 (77.3)	10 (22.7)	0 (0)
Penicillins	Piperacillin 100µg	0 (0)	18 (47.3)	20 (52.6)	16 (36.4)	28 (63.6)	0 (0)
Carbapenems	Meropenem 10µg	38 (100)	0 (0)	0 (0)	ND	ND	ND
	Imipenem 10µg	4 (10.5)	34 (89.5)	0 (0)	32 (72.7)	12 (27.3)	0 (0)
	Ertapenem 10µg	ND	ND	ND	44 (100)	0 (0)	0 (0)
Aminoglycosides	Gentamicin 10µg	22 (57.9)	16 (42.1)	0 (0)	10 (22.7)	20 (45.5)	14 (31.8)
	Kanamycin 30µg	ND	ND	ND	0 (0)	42 (95.5)	2 (4.5)
Tetracyclines	Tetracycline 30µg	38 (100)	0 (0)	0 (0)	44 (100)	0 (0)	0 (0)
Fluoroquinolone	Ciprofloxacin 5µg	8 (21.1)	6 (15.7)	24 (63.1)	28 (63.6)	14 (31.8)	2 (4.5)

Legend: R-Resistant; I- Intermediate; S- Sensitive; ND-Not determined

Table 4a. Multidrug-resistant profile of *E. coli* isolates from captive animals

Antimicrobial class	Antibiotics	Resistant phenotype	Number of isolates n=38	Multiple antibiotic-resistant index
6	9	CPM^R, CAZ^R, CXM^R, ATM^R, MEM^R, IMI^R, GEN^R, TET^R, CIP^R	3 (7.89)	0.75
6	8	CPM^R, CAZ^R, CXM^R, ATM^R, MEM^R, GEN^R, TET^R, CIP^R	6 (15.79)	0.66
5	7	CPM^R, CAZ^R, CXM^R, ATM^R, MEM^R, GEN^R, TET^R	17 (44.74)	0.58
3	5	CPM^R, CAZ^R, CXM^R, MEM^R, TET^R	30 (78.95)	0.42
3	4	CPM^R, CXM^R, MEM^R, TET^R	35 (92.11)	0.33
3	3	CPM^R, MEM^R, TET^R	38 (100)	0.25

Legend: CTX: Cefotaxime, **CPM**: Cefepime, **CTR**: Ceftriaxone, **CAZ**: Ceftazidime, **CXM**: Cefuroxime, **ATM**: Aztreonam, **PIP**: Piperacillin, **MEM**: Meropenem, **IMI**: Imipenem, **GEN**: Gentamicin, **TET**: Tetracycline, **CIP**: Ciprofloxacin.

Table 4b. Multidrug-resistant profile of *Salmonella* species from captive animals

Antimicrobial class	Antibiotics	Resistant phenotype	Number of isolates n=44	Multiple antibiotic resistant index
7	10	CTX^R, CPM^R, CTR^R, ATM^R, PIP^R, IMI^R, ETP^R, GEN^R, TET^R, CIP^R	9 (20.46)	0.91
6	8	CTX^R, CPM^R, ATM^R, PIP^R, IMI^R, ETP^R, TET^R, CIP^R	14 (31.82)	0.73
5	7	CTX^R, CPM^R, ATM^R, IMI^R, ETP^R, TET^R, CIP^R	22 (50.0)	0.64
4	6	CTX^R, CPM^R, ATM^R, IMI^R, ETP^R, TET^R	29 (65.91)	0.55
3	4	CTX^R, CPM^R, ETP^R, TET^R	38 (86.36)	0.36
3	3	CPM^R, ETP^R, TET^R	44 (100.0)	0.27

Legend: CTX: Cefotaxime, **CPM**: Cefepime, **CTR**: Ceftriaxone, **ATM**: Aztreonam, **PIP**: Piperacillin, **IMI**: Imipenem, **ETP**: Ertapenem, **GEN**: Gentamicin, **KAN**: Kanamycin, **TET**: Tetracycline, **CIP**: Ciprofloxacin.

DISCUSSION

The elucidation of disease-causing organisms from captive animals is significant for implementation of preventive, control and surveillance programs of diseases, as well as for implementing public and animal health policies (Jardine *et al*., 2011). The incidence of salmonellosis and toxigenic *E. coli* in individuals despite public health education has remained unchanged in the last 2 decades, with a shift in serotype dissemination from foodborne-associated strains towards environmentally-distributed strains, from which the routes are not always recognized (CDC, 2014). The present study has determined the population count and antimicrobial resistance profile of *Escherichia coli* and *Salmonella* species detected from the faeces of some captive animals in Ogba zoo, Benin City, Nigeria. The infectious dose of *Salmonella* has been reported to be > 10^5 while that of *E. coli* have been described to be between 10^6-10^8 organisms respectively. However, the infection may also be established at lower densities (Hara-Kudo and Takatori, 2011). The presence of high densities of the *Salmonella* and *E. coli* observed in this study coupled with the antibiotic-resistant index of the isolates establishes captive animals as a potential reservoir of pathogenic and antibiotic-resistant *Salmonella* and *E. coli* in the environment. Both disease-causing agents have been detected from several species of captive and wildlife origin (Hassan and Shobrak, 2014; Suphoronski *et al*., 2015; Hayashimoto *et al*., 2016; Hernandez *et al*., 2016). *Salmonella* has been elucidated from apparently healthy zoo animals (Stirling *et al*., 2008), and has also been associated in disease outbreaks with significantly elevated mortality in humans (Chomel, 2007; Marchant *et al*., 2016).

High level of prevalence of *Escherichia coli* and *Salmonella* species observed in the study, could be as a result of quality and storage of raw animal feeds, direct feeding by visitors, confinement conditions, contact with other captive animals and access of synanthropic wild animals (birds and rodents) (Conrad *et al*., 2017). A study by Saleha *et al*. (2015) in Malaysia revealed that 27.5 - 61.0% snakes, 28.0% chelonians, 26.5 – 36.0% lizards and 31.0% house geckos harboured *Salmonella*. Hidalgo-Vila *et al.* (2007) reported an incidence of *Salmonella*e in land-dwelling turtles in Spain to be 100.0% compared to aquatic turtles (in streams and ponds,) which were between 12.0-15.4%. Boey *et al*. (2014) reported occurrence in captive lizards from zoological parks and pet shops in Malaysia to be 83.0% as likened to wild ones (in and around domiciliary areas) to be 25.0%. Lukac *et al*. (2015) revealed a study from apparently healthy reptiles, privately owned or at a zoo in Croatia to be 13.0% positive for *Salmonella*, 48.0% in lizards, 9.0% in snakes and 4.0% in turtles. Callaway *et al*. (2011) reported an occurrence of *Salmonella* in the intestinal subjects to be 7.0% from Asian house gecko in Australia. Chambers and Hulse (2006) reported that 95.3% of turtles and snakes investigated in the USA were *Salmonella* positive. These findings are somewhat similar to the findings of this study as all captive animals in this study harboured *Salmonella* species and *E. coli* in their faeces.

Literature have demonstrated a direct correlation between the prevalence of *Salmonella* shedding in captive animals and their proximity to anthropogenic activities/habitats (Une *et al*., 2008; Daisuke, 2013). Urbanized animals have exclusive opportunities for exposure to enteric pathogen, infection, and dissemination resulting from various factors such as the quality of food and water they ingest, their comparatively sedentary lifestyles, the diversity of species with which they establish direct contact, as well as their consistent aggregations of elevated densities in small areas (Bradley and Altizer, 2007). Captive animals with a potential to be carriers of *Salmonella* and *E. coli* should be of significant interest due to their persistent contact with the public as well as their potential to contaminate environments inhabited by people.

With respect to the momentous temporal and spatial heterogeneity in antimicrobial resistance dissemination and the dynamics that upset its evolution, spread, and doggedness, it is imperative to pinpoint that antimicrobial resistance be seen as an ecological menace. Monitoring the prevalence of resistance of bacteria such as *Escherichia coli* and *Salmonella* in captive animals makes it conceivable to reveal that captive animals have the possibility to aid as a mixture and environmental reservoir of bacterial resistance. Hence it is important to address the issue of antimicrobial-resistant microorganism proliferating in the ecosystem and the environmental impact as well as related potential human health (Radhouani *et al*., 2014).

The incidence of antimicrobial resistance in captive animals has numerous implications which include the prospect to serve as an ecological reservoir of bacterial resistance; the zoonotic prospect of enteric bacteria; and the potential drawbacks of the medical treatment of captive animals. The occurrence of antibiotic-resistant pathogenic *Salmonella* and *E. coli* is of public health principal concern globally predominantly in relation to the failure of the treatment regimen. Several literatures have showed an increase in the occurrence of antibiotic-resistant *Salmonella* and *E. coli* and the upsurge of multidrug-resistant strains in humans and animal populations with more than 80.0% isolates resistant to sulphonamides, tetracycline, chloramphenicol and streptomycin (Radhouani *et al*., 2014; Saleha *et al*. 2015; Ones-Dias *et al*., 2016). Reports of Abatcha *et al*. (2013) also showed resistance to cephalothin, cephalexin, and amoxicillin-clavulanic acid from captive animals. Fazhana *et al*. (2007) revealed that all isolates from zoo animals were resistant to erythromycin, while 50.0% of the isolates were resistant to ampicillin and streptomycin. Nur-Mahiza *et al*. (2007) reported that 50.0% of the isolates were resistant to polymyxin B, followed by streptomycin and neomycin. Findings from these studies are similar to the findings in our present study. High multiple antibiotic resistant indexes observed in this study establishes the environments as a high-risk reservoir of antibiotic resistance. Hence, infections in humans emanating from such resistant salmonellae and *E. coli* in animals could be problematic and thus require stringent monitoring.

CONCLUSION

The present study has described the distribution and susceptibility profile of *Salmonella* species and *Escherichia coli* from captive animals in Ogba Zoological Garden. The presence of these potential pathogens coupled with their multidrug resistance potential call for public health concern. Further studies are required for the molecular characterization of the isolates to provide definitive evidence of resistance and virulence signatures coupled with zoonotic potential. The detection of these Enterobacteria suggests a significant risk of transmission between captive animals and close contacts, highlighting the necessity for staff training, improvement of hygienic procedures, public education and awareness of risks targeting visitors to chiefly utilize both educational and recreational activities of the catchment environment.

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Prevalence of Human Genital *Ureaplasma* sp. in a Cohort of Subjects in Southern and Northern, Nigeria.

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Abstract

Ureaplasma sp. can be found on the mucosal surfaces of the cervix or vagina of 40 to 80% of sexually matured women and implicated in several complications from urethritis to miscarriages. This study was thus aimed at ascertaining the prevalence of genital *Ureaplasma* sp. in Southern and Northern, Nigeria. Qualitative case controlled study was carried out in two different cities in Nigeria, while convenience sampling and a closed ended questionnaire where used to obtain data from 824 subjects. Triplicate urogenital swabs were taken from participants and transferred into transport and culture media. Specimens were inoculated onto A7 agar incubated at 37^{o}C, for 5 days in 5% CO_2. Cultures were examined microscopically daily for 5 days for the appearance of typical mycoplasma colonies. Results showed that *Ureaplasma* sp. was isolated from 22 of 70 males (31.4%) at the Northern Nigerian location, and from 19 of 70 males (27.1%) at the Southern Nigerian location. In females, 83 of 156 (53.2%) swabs were positive at JUTH, and 50 of 104 (48%) at LUTH. At the two locations, a total of 324 asymptomatic participants were sampled, and 28(8.6%) were positive. The prevalence rate of *Ureaplasma* sp. among the sexually transmitted disease (STD) patients was 24% while a value of 3.9% was recorded for the STD controls. Risk factors associated with genital *Ureaplasma* sp infection among the subjects include subjects with STI (p value=0.030), those with multiple number of sexual partners (p value=0.040), lack of the use of condoms (p value=0.014), age of sexual debut <18 years of age (p value=0.023) subjects with low socioeconomic status in occupation (p value=0.020), and level of education (p value=0.025). The association of genital mycoplasmas infections was strongest in participants <40 years of age (p value=0.059).

Key words: Sexually transmitted infections, Demographic information, Ureaplasma, Risk factors

Introduction

Mycoplasma and Ureaplasma species have been reported to be important etiological agents in urogenital ureaplasmosis (CDC, 2002). The three species that have been frequently isolated from human genitourinary tracts include *Ureaplasma* biovars *urealyticum* and *parvum*, *Mycoplasma hominis*, *Mycoplasma genitalium* (Casin *et al.*, 2002; Kiche *et al.*, 2004). These species have been confirmed to have a nexus with several complications in infected individuals including non-gonococcal urethritis in men, miscarriages, still birth and fetal defects in pregnant women as well as infertility in men and women. Urogenital ureaplasmosis have been identified in both sexually and non-sexually active individuals (Chukwuka *et al.*, 2013). The epidemiology and pathogenic roles of genital mycoplasmas in perinatal and neonatal infections in humans are fairly studied and understood globally; but it has been established that *Ureaplasma* sp. can be found on the mucosal surfaces of the

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cervix or vagina of 40 to 80% and *M. hominis* may occur in 21 to 53% of sexually matured women (Taylor-Robinson *et al*., 2005). Sexually matured persons with no history of sexual contact are infrequently colonized with genital mycoplasmas, even among those who are sexually experienced, colonization increases with increase in the number of different sexual partners. It is interesting to note that colonization increases more rapidly with increasing sexual experience in women than in men, suggesting that women are more susceptible to colonization with these organisms (Taylor-Robinson *et al.*, 2003).

In Nigeria several risk factors including age, occupation, gender, number of sexual partners, sexual frequency, level of education, the use of barrier cautions, such as condom and intrauterine devices (IUDs) have been associated with sexually transmitted diseases (STD) (Olorunshola *et al.*, 2007), but to the best of our knowledge, it appears no study has specifically addressed the prevalence and the risk factors associated with the transmission of human urogenital mycoplasmas which could form an epidemiological data for several locations in Nigeria. With the recent adoption of molecular characterization, the sensitivity and specificity in the identification of these species have increased.

The aim of this research was to create an epidemiological data for urogenital ureaplasmosis across selected locations in Nigeria through a survey in major STD centres within the country. We also evaluated the possible risk factors associated with the ureaplasmosis through the use of structured questionnaires.

Materials and methods

Study design

Study design employed was a descriptive epidemiological study involving 5 study centers (3 in Lagos University Teaching Hospital (LUTH) and 2 in Jos University Teaching Hospital (JUTH). LUTH site involved Departments of Obstetrics, Gynecology and Surgery (Cytology and Urology units) as well as Microbiology diagnostic unit; while Special Treatment Clinics and Microbiology diagnostic unit were involved at JUTH site. The two teaching hospitals serve as the Federal Government's Reference Centers for HIV/AIDS /STD in the Southern and Northern parts of Nigeria, respectively.

Study population

The study involved STI patients who presented with discharge, itching, ulcerations and sores in genital parts, those who had abortion, as well as patients with various degrees of infertilities attending STD clinics at Lagos University Teaching Hospital (LUTH) and Jos University Teaching Hospital (JUTH). The asymptomatic patients (controls) were subjects accompanying STD patients attending antenatal, prenatal, or postnatal clinics, as well as individuals referred by employers for medical tests or religious leaders for HIV screening recommended during marriage counseling.

Samples size and sampling method

The qualitative case controlled study as described by Schlesselman (1982) was employed in the two metropolitan cities' hospitals in the Northern and Southern Nigeria. The study aimed at investigating the relationship among the studied genital mycoplasmas and reproductive maladies such as urethritis, cervicitis, pelvic inflammatory diseases, spontaneous abortion and various degrees of infertility among male and female patients attending STI clinics in Nigeria. A non-probability sampling method, known as convenience sampling method, was used. The sample size was determined using the formula recommended by WHO (2001a). LUTH Site's breakdown included 70 males with STI symptoms, 180 females with STI symptoms, 70 asymptomatic males and 92 asymptomatic females amounting to 412 participants. JUTH Site's Breakdown included, 70 males with STI symptoms, 180 females with STI symptoms, 70 asymptomatic males, 92 asymptomatic females amounting 412 participants all together.

Culture Media Preparation

Triplicate urogenital swabs were taken from participants referred to LUTH and JUTH. Samples were collected from patients with age ranging from 15 to 65 years. In order to maximize the growth of mycoplasmas both transport and culture media were prepared from PPLO broth supplemented with horse serum (20%), 10ml yeast extract (25%), 2ml phenol red (0.2%), penicillin (500u/ml), in 3 separate tubes containing 20ml urea (10%), for *Ureaplasma* sp.; 20ml L-arginine (10%) for *M. hominis* and *M. genitalium*; and 20ml glucose (10%) for *M. fermentance* and the pH of the substrates were adjusted to 6.0, 6.5 and 7.5

respectively. Urogenital swabs were transported in 5ml 2SP medium (Shepard and Luceford, 1976) on ice from the clinics to our laboratory. Specimens were inoculated onto A7 agar (Becton Dickinson, Cockeysville, Md21030), incubated at 37˚C, for 5 days in 5% CO_2. Cultures were examined microscopically daily for 5 days for the appearance of typical mycoplasma colonies. A7 agar incorporates a direct test for urease that allows the differentiation of Ureaplasma from other Mycoplasma (Shepard and Luceford, 1976).

Results

Subjects attending the study centers representing 30 out of the 37 states in Nigeria participated in the completion of the questionnaires at JUTH and LUTH centers. A total of 824 samples were collected from participants with age ranging from 11 to over 50 years. Table 1 shows the geographical distribution of the subjects. The percentage of younger participants (11-20yrs) recorded in the north was 54.2% as opposed the south which experienced participation from older subjects (31-40yrs) at 43.9% (Table 1).

The number of respondents was relatively higher in females at both sites. In females, 58.3% and 66.0% were recorded while in males, 41.7% and 34.0% for LUTH and JUTH, respectively (Table 1). More than 50% of the participants had no children per household at both sites, while 9.5 - 40.3% had 1 or more children. Subjects from the northern geographical zone had higher number of children per household compared to the southern zone (Table 1). Over 50% of the participants were unemployed; the number of the employed respondents was the lowest in the occupation category. In Table 2, a higher percentage of the respondents- 45.5% and 55.6% (JUTH and LUTH, respectively) had their first sexual contact at 16 – 20 years age bracket. Over 75% of the participants were also married while approximately 20% were single by being unmarried, widowed or divorced. About 85% of the participants at both sites reported mono-sexual partners, also a higher percentage responded to having one sexual partner in the last three months. In JUTH, 87% of subjects admitted previous diagnosis of STI which is considerably higher than 59.8% seen in LUTH. The practice of safe sex (use of condom) in the southern region was more pronounced (57.3%) compared to the northern region (38.3%). Both sites however, showed a considerably low rate of IUD use for birth control (Table 2).

Based on the residents and geographical location of the participants in this study, the prevalence rate of genital ureaplasmosis is presented in Figure 1. Of the 824 overall participants, 512 Subjects came from the northern region, while 312 were from the southern region; total number of patients was 500 while total number of control was 324. The distributions of participants covered 6 geopolitical zones of Nigeria (North East, North West, North Central, South West, South East and South-south). The highest prevalence among the patients was recorded in the North Central zone with a frequency of 144 out of 400 (36%); while the least prevalence was observed in the North West zone (6.25%). Also for the controls, the highest prevalence was recorded in the North Central zone with a frequency of 158 out of 324 (48.8%).

Table 3 presents the **s**ummary of the results of the primary isolation of *Ureaplasma* sp. from Southern (LUTH) and Northern (JUTH) Nigeria. *Ureaplasma* sp. was isolated from 22 of 70 males (31.4%) at the JUTH location, and from 19 of 70 males (27.1%) at the LUTH location. In females, 83 of 156 (53.2%) swabs were positive at JUTH, and 50 of 104 (48%) at LUTH. At the two locations, a total of 324 asymptomatic participants were sampled, and 28(8.6%) were positive. The prevalence rate of *Ureaplasma* sp. among the STD patients was 24% while a value of 3.9% was recorded for the STD controls. Overall, 27.9% (202 of 724) of the total participants were positive for *Ureaplasma* sp. colonization/ infection.

. **Table 1. Distribution of correspondence by Zone**

		Patients		**Controls**		**Total**	
s/no	**Zones**	**Frequency**	**Percent**	**Frequency**	**Percent**	**Frequency**	**Percent**
1	North East Zone	38	7.6	22	6.8	60	7.3
2	North West Zone	25	5.0	25	7.7	50	6.0
3	North Central Zone	244	48.8	158	48.8	402	48.8
	Sub total	**307**	**61.4**	**205**	**63.3**	**512**	**62.1**
4	South West Zone	59	11.8	33	10.2	92	11.2

5	South East Zone	92	18.4	50	15.4	142	17.2
6	South South Zone	42	8.4	36	11.1	78	9.5
	Sub total	**193**	**38.6**	**119**	**36.7**	**312**	**37.9**
	Gross total	500	100.0	324	100.0	824	100.0

Table 2: Demographic characteristics of the respondents by Teaching Hospitals

Characteristics	**Percentage of respondents**		
	JUTH	**LUTH**	**P value**
Age groups of the respondents			P = 0.059
11—20	54.2	35.9	
21—30	35.7	32.9	
31—40	39.0	43.9	
41--50+	12.3	23.2	
Sex			
Female	66.0	58.3	
Male	34.0	41.7	
No of children per family			
0	59.7	90.5	
1 and above	40.3	9.5	
Occupation			0.020
Unemployed	59.7	50.0	
Self-employed/Residual Category/Artisan	25.3	22.0	
Employed	15.0	28.0	
Level of education			0.025
Tertiary	37.0	42.7	
Secondary	33.8	45.1	
Primary	29.2	12.2	
Source of referral			
Counselor/Friend/self	34.4	14.2	
Dr.	42.9	81.0	
Employer	22.7	4.8	

Table 3: Risk factors of human genital Ureaplasma amongst the respondents

Characteristics	**Percentage of respondents (%)**		
	JUTH	**LUTH**	**P value**
Age of first sexual contact			0.023
11—15	16.0	6.1	
16—20	45.5	55.6	
21—25	20.2	25.4	
26—30 and above	18.3	12.9	
Marital status			
Married	84.4	79.3	
Single	15.6	20.7	
No. of sex partner			

1	83.8	83.3	
2 and above	16.2	16.7	
Sexual partner in the past 3 months			0.040
0	17.5	39.0	
1	72.1	58.5	
2 and above	10.4	2.4	
History of STI			0.030
No	13.0	40.2	
Yes	87.0	59.8	
Use of condom			0.014
No	61.7	42.7	
Yes	38.3	57.3	
Use of IUD			
No	78.6	79.3	
Yes	21.4	20.7	

Table 3: Prevalence of *Ureaplasma* sp. among the subjects

Prevalence	**JUTH**				**LUTH**			
	Control		**Patient**		**Control**		**Patient**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
No. examined	70	116	70	156	60	78	70	104
No. of positive	5	8	22	83	9	6	19	50
Rate (%)	7.1	6.9	31.4	53.2	15	7.7	27.1	48

Discussion

From this study, epidemiological data on the incidence and prevalence of genital mycoplasma in the northern and southern regions of Nigeria were obtained. The predisposing factors to colonization or infection by genital mycoplasma were also assessed in the work. Generally, more females participated in the study at both study cente

rs which probably could be attributed to the increased susceptibility of females to STIs. In the study center representing the northern region (JUTH), it was discovered that a higher percentage (54.2%) of the participants from the study sites were relatively adolescents and young adults. Despite the young age bracket, 84.4% of the respondents are married and sexually active. This is in contrast to the respondents at LUTH, where the larger number belonged to 31 - 40 years of age with 79.3% being married. The higher number observed in JUTH could be as a result of the cultural and religious ethics of early marriage that is peculiar to the northern region.

Most of the subjects used in this study (from both study sites) also had their first sexual contacts between the age of 16 and 20 years. It was observed that over 80% of the participants at both study sites had just one sexual partner. The low number of multiple sexual partners could be significant in reducing the spread of STIs or mitigate the chances of contracting it. However, despite the high number of mono sexual partners, there was a relatively high report of previous cases of STI in this research. From the northern study center, 87% of the participants had at one time been diagnosed of STI while 59.8% followed suit at the southern study site. The use of condoms for safe sex practice as well as the use of IUD for birth control was low at JUTH; more participants used condom for safe sex practice at LUTH but the use of IUD was also relatively low. The high percentage of STI history especially in the northern region could be attributed to the low practice of safe sex which increases chances of contracting sexually transmitted infections.

The overall prevalence of genital mycoplasma is 27.9% (11.6% for LUTH; 16.3% for JUTH). It was

also observed that mycoplasma infection was more pronounced in females than in males in both study centers. Prevalence of genital mycoplasma was higher in females (20.3%) than in males (7.6%). This suggests that females are more susceptible to colonization or infection by mycoplasma or they are more symptomatic than males. However, there have been reports of higher prevalence from Ibadan (35.7%) (Agbakoba *et al.,* 2008) and northern Nigeria (32.5%) (Jombo *et al.,* 2009). The lower prevalence recorded in this study could be as a result of the population used could be as a result of single sexual partners which was highly reported by the respondents. %). The rate of isolation of *Ureaplasma* sp. was shown to be higher in patients (43.5%) with identified sexual risk behaviors than in asymptomatic controls (8.6%). This current study has also confirmed the presence of genital mycoplasma in Nigerians suffering from obstetrics and gynecological problems, utilizing the facilities at JUTH and LUTH, Nigeria. Our overall findings demonstrated a strong association between certain risk factors and detection of mycoplasmas, suggesting that infections could become endemic if control measures are not put in place.

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