PUBLIC HEALTH SIGNIFICANCE AND ANTIBACTERIAL ACTIVITIES OF PEPPER (*CAPSICUM ANNUM*) AND ONION (ALIUM CEPA) ON ISOLATES FROM URINARY TRACT INFECTIONS

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Abstract
This study assessed the antimicrobial activities of aqueous extracts of *Capsicum annum* (pepper) and *Allium cepa* (onion) of 30 isolates of urinary tract infections. Isolation and identification of the bacteria species were carried out using standard microbiological and biochemical techniques. Kirby Bauer disc diffusion technique was employed for the antibiotic study using relevant Gram-positive and Gram-negative antibiotic discs. Zones of inhibition were compared with the guidelines set by the Clinical Laboratory Standard Institute (CLSI). Percentage prevalence showed that *Escherichia coli* (30%) had the highest occurrence followed by *Proteus mirabilis* (20%), *Staphylococcus aureus* (13%), *Streptococcus* sp. (12%), *Bacillus* sp. (8%), *Citrobacter freundii* (6%), *Klebsiella pneumonia* (6%) while the least dominant was *Neisseria* sp. (5%).The antimicrobial effects of aqueous extract of pepper on the isolates showed that the highest zone of inhibition for all the isolates was at 100 mg/ml It was observed that virtually all the tested drugs showed high level of susceptibility to the bacterial isolates such as ciprofloxacin (100%), pefloxacin (100%) and augmenting (100%) while the rest antibiotics were above 50% effectivity. The study also revealed that all tested antibiotics were equally susceptible to the Gram-negative bacteria isolates such as ciprofloxacin (100%), nefloxacin (100%), septrin (100%), rifampicin (100%), erythromycin (100%), augmentin (100%) and levofloxacin (100%). Thus, the findings suggest that aqueous extracts of onion and pepper at 100 mg/ml concentrations have antibiotic properties.

Keywords: Onion, Pepper, Antimicrobial properties, Urinary tract infections

INTRODUCTION

Urinary Tract Infections (UTI) is an infection caused by the presence and replication of microorganisms in the urinary tract. It is the single most common bacterial infection of mankind (Ebie *et al*., 2011; Morgan and McKenzie, 2013). Females are believed to be more affected than males except at the extremes of life (Kolawole *et al*., 2009). This is because bacteria can reach the bladder more easily in women, partially due to the short and wider female urethra, and its proximity to the anus. Available scientific information indicates that bacteria easily travel up to the urethra from the rectum and thereby causing infection (Ebie *et al*., 2011). Urinary tract infection is one of the most common bacterial infections and Gram negative bacteria are among the most prevalent bacteria detected from urinary tract patients. The organism is of clinical importance due to its cosmopolitan nature and ability to initiate, establish and cause various kinds of infections. It is one of the most frequently isolated organisms from different clinical cases of diarrhea. More than 50% of urinary tract infections in patients are accounted for *Escherichia coli* (Selvarangan *et al*., 2014). The predominance of *Enterobacteriaceae* and particularly *Escherichia coli* remain the principle pathogen causing Urinary tract infection, accounting for 75-90% of all UTI's in both inpatients and outpatients (Nerurkar and Shanta, 2012). In addition, *Klebsiella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Proteus* spp., *Pseudomonas* spp. and *Enterococcus* spp. were more often isolated from inpatients (Barisic *et al*., 2013). Elsewhere, coagulase negative *Staphylococci* may be a common cause of urinary tract infection in some reports, whereas anaerobic organisms are rarely pathogens in the urinary tract (Mandell *et al*., 2005). The beta-lactam antibiotics have been widely used since 1980 for the treatment of serious infections caused by gram- negative bacteria, but resistance against these antibiotic groups occurred quickly worldwide (Gniadkowski, 2011). Herbal remedies are widely known to be used in the treatment of many infectious microorganisms. Plant materials continue to provide a major source of natural therapeutic remedies and play an important role many health care system of developing countries (Malomo *et al*., 2006; Onyije *et al*., 2012; Osuagwu *et al*., 2007). Traditional healers have long used plants and plant products to prevent or cure infectious conditions and other diseases in man (Fowotade *et al*., 2017; Oduola *et al*., 2010). Western medicine is trying to duplicate their successes (Purseglove, 2015; Dare *et al*., 2013). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties (Cowan, 2011). However, since many of these compounds got from plants material are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, it is therefore necessary to consider the consequences (positive or negative) of patients' self-medication with these preparations and to confirm the potency of such preparations. Onion and pepper are good examples of such plants, which have been found out to be used locally against infectious, particularly in some cases of eye infections (Cowan, 2011). Onion bulbs contain a good number of phytochemicals, most of which are hydrocarbons and their derivatives. These include: Dipropyl disulphide, allicin, diathyl sulphide, dimethyl disulphide, Mercaptopropane or propylmercaptan (Jeffrey and Herbert, 2013). Several parts of the plant have traditional place in folk medicine in many countries and the extracts from onion and garlic, which is another species of Allium, have antimicrobial properties (Purseglove, 2015). Crude juices of onion and garlic bulbs exert inhibition on the growth of *E. coli*, *Pseudomonas pyocyaneus*, *Salmonella typhi*, *Bacillus subtilis*, in vitro (Abdou *et al*., 2011). Pepper (*Capsicum*), fruit and vegetables are important sources of bioactive compounds (such as phenolic compounds, terpenoids, steroids and alkaloids) known for their health-promoting effect against degenerative diseases. Due to increase in resistance of pathogens to most synthetic drugs, it became necessary to assess the antimicrobial activities of aqueous extracts of Capsicum annum (pepper) and Allium cepa (onion) on isolates of the urinary tract infection for possible treatment of diseases and infections.

MATERIALS AND METHODS

Study Area

The study was conducted in Abraka, in Ethiope East Local Government Area, Delta State. Abraka. Abraka is located 5^0 47′ 0″ North and $6^0$6′0″ East of Delta State, Nigeria.

Sample collection

This was carried out using procedure reported in Cheesebrough (2004). A total of 30 samples (mid-stream urine) were collected from five (5) different laboratories in Abraka, Delta State into sterile universal containers. They were labelled and were immediately transferred to the laboratory for analysis. The urine samples (10 ml) were collected in a centrifuge tube and were spun at 1500 rpm for 5 minutes. The supernatant were decanted and the deposits were observed under the microscope, low power for pus cells, epithelial cells, cast cells, crystals and yeast cells (Cheesebrough, 2006). Collection of plant material. The onion and pepper samples used in this study were obtained from vendors in an Abraka market. The onion and pepper samples obtained were then peeled and sun dried.

Extract preparation

Extracts of onion and pepper were prepared in accordance with the procedure reported by Food and Agricultural Organization, FAO (1996). The plant samples were sun-dried sufficiently and blended into a fine powder. Twenty-five (25 g) of the powder were weighed and suspended in 125 ml each of distilled water. The extraction was allowed to proceed at room temperature for up to 120 hours. Extracts were decanted and filtered through a whatman filter paper. Filtrate was gently evaporated to dryness at 45^{o}C. Residue obtained were reconstituted in 95% ethanol at a stock concentration of 0.2 g/ml and refrigerated until they were required for use.

Inoculation of Samples

After the preparation of media (CLED, Nutrient and MacConkey agar), they were dispensed into sterile petri dishes and allowed to solidify, The urine samples were inoculated on both MacConkey, CLED Agar and nutrient agar, which were incubated at 37°C for 24 hours using the streak plate technique. A loop full of each urine sample was inoculated into the various media and incubated for 24 hours at 37°C. Isolates were identified on the basis of Gram staining techniques, motility test and relevant biochemical techniques such as indole, citrate, oxidase, catalase, triple sugar ion test.

Sensitivity test using plant extracts

Plant extracts were serially diluted at 100, 50, 25 and 12.5 mg/ml concentrations. A freshly prepared Muller Hilton agar was poured into a sterile petri dish and allowed to cool. A sterile swab stick was used to collect microorganism already grown in the peptone water in a test tube. The sterile swab stick was used to swab the surface of the Muller Hilton agar. 0.1 ml of the diluents were withdrawn from each test tube and introduced into the holes in the agar and incubated for 24h at 37 ^{o}C.

Antibiotic Sensitivity testing

This was carried out in accordance with the procedure reported by Kirby-Bauer disc diffusion method (1997). The organisms were cultured in peptone water for 5 – 6 hrs. Thereafter, a sterile swab stick was used to pick bacterial inoculum corresponding to 10^{5}cfu/ml (0.5 McFarland standard) that grew on the peptone water and was used to swab the surface of the nutrient agar plates. The antibiotic discs were placed on the surface of the nutrient agar and were incubated at 37 ^{o}C for 24 hrs. If the growth of the organism is inhibited by the action of the antibiotic it is reported to be sensitive to the antibiotic. If the organism is not susceptible to the antibiotic it is reported to be resistant to the antibiotic. Zones of inhibition $\leq$ 12 mm were recorded as resistant (R), while zones of inhibition > 12 mm were recorded as sensitive (S). The zone of inhibition shows how susceptible an organism is to the antibiotic. It should also be noted that too heavy an inoculums reduces the size of the inhibition zone. On the other hand, too light an inoculums makes it difficult to read the zones of inhibition correctly. The inhibition zones were measured and interpreted by the recommendations of the Clinical Laboratory Standard Institute (CLSI, 2006).

RESULTS

The total bacterial count of the urine samples from five different laboratories in Abraka, Delta State is presented in Table 1. It was observed that Lab D had the highest bacterial count of 5.32 ± 0.11 x 10^4Cfu/ml while Lab B had the least bacterial count of 3.4 ± 0.30 x 10^4Cfu/ml. The recorded that *Escherichia coli* (30%) was the most prevalent isolate while the least was *Neisseria* sp. (5%) as shown in Table 2. The antimicrobial effects of aqueous extract of pepper on the isolates is revealed in Table 3. It was observed that the highest zones of inhibition for all the isolates was at 100 mg/ml concentration of the aqueous extract of pepper while there were no zones of inhibition at 12.5 mg/ml concentration of the aqueous extract of pepper. *Bacillus* sp had the highest zone of inhibition (12 mm) at 100 mg/ml concentration. The antimicrobial effects of aqueous extract of onion on the isolates is presented in Table 4. At 100 mg/ml concentration, *Proteus mirabilis* had the highest zone of inhibition of 12mm while *Bacillus* sp. had the least zone of inhibition of 8mm. At 50 mg/ml concentration, *Proteus mirabilis* and *Streptococcus* sp had the highest zones of inhibition of 9 mm each while *Bacillus* sp had no zone of inhibition. There were no zones of inhibition (0 mm) observed at 12.5 mg/ml concentration of the aqueous extract of onion sample used. The zones of inhibition of Gram negative isolates to commercially available antibiotics and the percentage susceptibility profile showed that most tested antibiotics were sensitive to the isolates as presented in Table 5. The zones of inhibition of Gram positive isolates to commercially available antibiotics are shown in Table 6. It was observed that most of the antibiotics were sensitive to the bacterial isolates, when compared with the CLSI guideline (2016)

Table 1: Total bacterial count of the urine samples (x10^3)

Location	Plate count (Cfu/ml)
Lab A	4.80 ± 0.22
Lab B	3.41 ± 0.30
Lab C	4.20 ± 0.41
Lab D	5.32 ± 0.11
Lab E	3.90 ± 0.28

Key**:** Lab A = Medisaj lab, Lab B = Winners lab, Lab C = Divine Medicare Lab, Lab D = Emma Maria Lab, Lab E = Emmanuel Lab.

Table 2.Frequency by occurrence of bacterial isolates

Bacteria	%
Proteus mirabilis	20
Neisseria sp	5
Citrobacterfreundii.	6
Bacillus sp.	8
*Streptococcus*sp	12
Esherichia coli	30
Staphylococcus aureus	13
Klebsiella pneumonia	6

Table 3: Antibacterial effects of aqueous extract of pepper on the isolates

	Zones of inhibition (mm)			
Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
Proteus mirabilis	10	9	8	0
*Neiserria*sp.	9	6	0	0
Citrobacterfruendi	11	9	0	0
Bacillus sp.	12	8	5	0
Streptococcus sp.	9	0	0	0
Klebsiella pneumonia	10	6	0	0
Staphylococcus aureus	10	10	0	0

Table 4: Antibacterial effects of aqueous extract of onion on the isolates

	Zones of inhibition (mm)			
Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
Proteus mirabilis	12	9	6	0
*Neiserria*sp	9	6	0	0
Citrobacterfruendi	9	6	0	0
Bacillus sp	8	0	0	0
*Streptococcus*sp	10	9	0	0
Klebsiella pneumonia	11	7	0	0
Staphylococcus aureus	10	6	0	0

Table 5: Zones of inhibition (mm) of gram negative isolates to antibiotics

Isolates	CPX	SXT	S	APX	CEP	OFX	NA	PEF	CN	AU
Proteus mirabilis	25 (S)	25 (S)	18 (S)	18 (S)	15 (S)	25 (S)	15 (S)	25 (S)	25 (S)	25 (S)
Neisseria sp	25 (S)	20 (S)	20 (S)	25 (S)	25 (S)	25 (S)	25 (S)	25 (S)	25 (S)	25 (S)
Citrobacterfreundii	25 (S)	6 (R)	7 (R)	5 (R)	12 (R)	10 (R)	12 (R)	15 (S)	10 (R)	15 (S)
Escherichia coli	25 (S)	0 (R)	25 (S)	6 (R)	25 (S)	25 (S)	0 (R)	25 (S)	8 (R)	25 (S)
Klebsiella pneumonia	25 (S)	25 (S)	25 (S)	25 (S)	25 (S)	25 (S)	15 (S)	25 (S)	25 (S)	25 (S)
	100%	60%	80%	60%	80%	80%	60%	100%	60%	100%

Key: SXT = septrin (25µg), CPX = ciprofloxacin (5 µg), S = Streptomycin (10 µg), APX = ampiclox (10 µg), CEP = ciprofloxacin (10 µg), ofloxacin (30 µg), NA = nalixodic acid (30 µg), PEF = pefloxacin (10 µg), CN = gentamycin (10 µg), AU = augmentin (10 µg), S = sensitive, R = resistant.

Table 6: Zones of inhibition (mm) of gram positive isolates to antibiotics

Isolates	CIP	NEF	CN	AM	S	RF	E	CH	AM	L
*Bacillus*sp	25 (S)	25(S)	25(S)	15 (S)	15(S)	15(S)	25(S)	25(S)	25(S)	25(S)
Streptococcus sp	25(S)	15(S)	25(S)	10(R)	15(S)	25(S)	25(S)	10(R)	15(S)	25(S)
Staphylococcus sp	15(S)	15(S)	25(S)	15(S)	15(S)	15(S)	25(S)	15(S)	25(S)	25(S)
	100%	100%	100%	67%	100%	100%	100%	67%	100%	100%

Key: CPX = ciprofloxacin (5µg), NFX = nefloxacin (10 µg), CN = gentamycin (10 µg), RF = Rifampicin (10 µg), E = erythromycin (10 µg), CH = chloramphenicol (10 µg), AM = ampiclox (10 µg), L = levofloxacin (5 µg), R = resistant, S = sensitive

DISCUSSION

Total bacteria count of urine samples from the five (5) different laboratories in Abraka, Delta State ranged from 3.4 ± 0.30 x 10^4 to 5.32 ± 0.11 x 10^4 CFU/mL. These counts supports the recommended standard of 10^4 - 10^5/ml set by Cheesebrough (2000) for possible cause of urinary tract infection. This might be due to poor hygienic practices and sexual intercourse. Bacterial species isolated from this study includes were *Proteus mirabilis, Neisseria* sp., *Citrobacter freundii, Bacillus* sp., *Streptococcus* sp., *Esherichia coli, Staphylococcus aureus and Klebsiella pneumonia*. Percentage prevalence showed that *Escherichia coli* (30%) appeared to be highest prevalent followed by *Proteus mirabilis* (20%), *Staphylococcus aureus* (13%), *Streptococcus* sp. (12%), *Bacillus* sp. (8%), *Citrobacter freundii* (6%), *Klebsiella pneumonia* (6%) while the least dominant was *Neisseria* sp. (5%). Presence of these organisms in the urine of patients studied indicate a potential source of urinary tract infection. These pathogens responsible for urinary tract infections may be endogenous, but there is possibility of infection been introduced from exogenous sources during diagnostic or therapeutic instrumentation. The organisms isolated were similar as those isolated in the work of Sibi *et al*. (2011) which were *Escherichia coli* (39.3%), *Staphylococcus* sp. (18.4%), *Klebsiella* sp. (15.7%) *Enterococcus* sp. (13.1%), *Proteus* sp. (7.8%), *Pseudomonas* sp. (2.6) and *Candida* sp. (2.6%).The isolates were tested for their susceptibility patterns to different concentrations of the aqueous extract of pepper (*Capsicum* *annuum*) obtained from shops in Abraka market, Abraka, Delta State. The result showed that the isolates were most sensitive to the pepper extract at 100mg/ml concentration and were least sensitive at 12.5 mg/ml concentration. Apart from the general use of pepper as a food ingredient, it has been reportedly used as a remedy for treating different diseases including gastroenteritis, stomach ache, diabetes, arthritis, pain etc (Anikwe *et al*., 2017). Ajayi *et al*. (2016) investigated the potential of Capsicum annum seeds which have been long known for the ethno-botanical uses, as a source of antimicrobials. Analysis of the seeds used in their study showed that the ethanolic extract had the highest antimicrobial effect on *Staphylococcus aureus* at 0.6 g/ml with the highest zone of inhibition at 18 mm. It was also observed that the ethanolic extract had the highest antimicrobial effect on *Streptococcus* species with the concentration 0.6 g/ml giving the highest zone of inhibition at 15.0 mm. The effect of aqueous extract of onion on the microorganisms showed that *Proteus mirabilis* had the highest zone of inhibition at 100 mg/ml and 50 mg/ml concentrations. There were no zones of inhibition (0 mm) observed at 12.5 mg/ml concentration. The bacterial isolates from urine sample were further exposed to commercially available antibiotics in order to compare the zones of inhibition with that of onion and pepper used. The percentage antibiotic susceptibility profile for the gram positive and gram negative bacterial isolates showed that most of the tested antibiotics were sensitive to the isolates and will therefore be recommended for possible treatment of urinary tract infections. In comparison with the medicinal plants used in this study, the commercially available antibiotics were more potent on the bacterial isolates than the medicinal plants. This report corroborates with the findings of Edoh and Mensah (2007) who also reported high level of susceptibility of the bacterial isolates to the antibiotics.

Conclusion

The findings of this study has indicated that microorganisms such as *Proteus mirabilis, Neisseria* sp., *Citrobacter freundii, Bacillus* sp., *Streptococcus* sp., *Esherichia coli, Staphylococcus aureus and Klebsiella pneumonia* were present in the urine of patients in the five different laboratories sampled and may be associated with urinary tract infection of the patients. The study showed that aqueous extracts of onion and pepper at 100% concentration is effective for the treatment of infections arising from the bacterial isolates. In addition, all the tested antibiotics equally showed great potential to the bacterial isolates, this should be recommended for possible treatment of urinary tract infection.

REFERENCES

Abdou, I.A., Abdouzeid, A.A., EI-Sherbeery, M.R. and Abdou, E. (2011). Nutrition, Cairo. Quality Plant. Mater Vegetable. 22(1): 29-35.

Anikwe, L.U.1., Onoja, U.S., Onyeke, C.C. and Nweze, E.I. (2017) Antimicrobial activities of four varieties of Capsicum annuum fruits cultivated in Southeast Nigeria against multidrug-resistant and susceptible organisms. *Journal of Basic Pharmacology and Toxicology*, 1(2):21-26.

Barisic, Z., Babic´-Erceg, A. and Borzic, E.l. (2013).Urinary tract infections in South Croatia: aetiology and antimicrobial. *International Journal of Antimicrobial Agents.* 22: 61-64.

Chesebrough, M. (2000). Medical Laboratory Manual for Tropical Countries Volume 11. Second edition, University Press, Cambridge, Great Britain, pp. 377-378.

Clinical and Laboratory Standard Institute (CLSI) (2016). Performance Standard for Antimicrobial Susceptibility Testing, twenty-fourth informational supplement, CLSI document M100-524, Wayne, P.A. United States. pp. 50-57.

Cowan, M.M. (2011). Plant products as antimicrobial agents. Clinical microbiology Reviews, 12(4): 564-582.

Dare, W.N., Igbigbi, P.S. and Avwioro, O.G. (2013). The Effect of Chronic Honey Intake on Sperm Parameters and Fertility Potential in Adult Male Wistar Rats. *World Applied Sciences Journal* 22 (5): 657-661.

Ebie, M. Y., Kandakai-Olukemi, Y. T., Ayanbadejo, J. and Tanyigna, K. B. (2011). Urinary Tract Infections in a Nigerian Military Hospital.Nigerian. *Journal of Microbiology*, 15(1): 31-37.

Edoh, D. and Mensah, A. (2007). Incidence of antibiotic resistant microbes in Acrra west, Ghana. *African Journal of Science and Technology*, **8**(2): 103-109

Fowotade, A.A., Fowotade, A., Enaibe, B.U. and Avwioro, O.G (2017). Evaluating Toxicity Profile of Garlic (Allium sativum) on the Liver, Kidney and Heart Using Wistar Rat Model. *International Journal of Tropical Disease andHealth* 26 (2): 1-12

Gniadkowski, M. (2011). Evolution and epidemiology of extended-spectrum beta-lactamases (ESBLs) and ESBL producing microorganisms. *Clinical Microbiology and Infectection.* 7: 597-608.

Jeffrey, B.H. and Herbert, B. (2013). Photochemical Dictionary. A hand book of bioactive compounds. 1st edition, Tavlor and Francis London Washington D.C. pp 234-245.

Malomo, A.O., Ekpo O.E., Imosemi I.O., Owoeye O., Osuagwu F.C., Avwioro O.G. and Shokunbi M.T. (2006) Neuroprotective effect of dexamethasone on the morphology of the irradiated postnatal developing cerebellum of wistar rat (rattus novegicus). *International Journal of Morphology* 24: 221-119

Mandell, G.L, Bennett, J.E. and Dolin, R. (2005).Principles and practice of infec-tious diseases. Churchill Livingstone, 881-882.

Morgan, M.G and McKenzie, H. (2013). Controversies in the Laboratory Diagnosis of Community Acquired Urinary Tract Infection. *European Journal of Clinical Microbiology*, 12 (7): 491-504.

Mueller, M., Hobiger, S. and Jungbauer A. (2010). Anti-inflammatory activity of extracts from fruits, herbs and spices. *Food Chemistry*122: 987– 996.

Nerurkar, A. P. and Shanta S. N. (2012). Bacterial pathogens in urinary tract infection and antibiotic susceptibility pattern. *Journal of Pharmaceutical and Biomedical Sciences*, 21: 32-35.

Oduola, T., Bello, I.S., Idowu, T.O., Avwioro, O..G., Adeosun, O.G and Isa, L.O.(2010). Histopathological changes in Wistar albino rats exposed to aqueous extract of unripe Carica papaya. *North American Journal of Medical Science* 2:(5); 234-237

Onyije, F.M., Avwioro, O.G., Atoni, A.D., Nduku, A. and Zenebo, V.C. (2012). Evaluation of the kidney following administration of unprocessed honey. *Asian Journal of Medical and Pharmaceutical Research*. 2(1): 21-25

Osuagwu, F.C., Owoeye, O., Avwioro, O.G., Oluwadara, O.O., Imosemi, I.O., Ajani, R.S., Ogunleye, A.A. and Oladejo, O.W. (2007) Reduction of Hippocampal CA 1 neurons in Wistar rats following the administration of phenytoin for seven days. *African Journal of Medicine and Medical Science* 36:103-108

Purseglove, J.W. (2015). Tropical crops [Monocotyledon]. Longman Scientific and Technical, England. Pp. 27-38.

Selvarangan, B.N. and Jasmine, R. (2014). Antibiotic susceptibility of ESBL-producing urinary isolates at a tertiary care hospital in Tiruchirappalli, South India. *Journal of Medical Science*. 7:443–446.