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RESEARCH ARTICLE **AJHSE050113**

ANTIMICROBIAL EFFECT OF CLOVES (*Syzygium aromaticum*) ON *Escherichia coli* FROM FEMALE ADULTS URINE WITHIN ALUU COMMUNITY, RIVERS STATE, NIGERIA.

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ABSTRACT

The study was aimed at determining the antimicrobial effect of clove on *Escherichia coli* isolated from the urine of female adults in Aluu community, Rivers State. Clean-void, mid-stream urine (MSU) specimens were collected from 30 female adults and 20 pregnant women residing in Aluu community, Port Harcourt Rivers state. The samples were culture on Eosin Methylene Blue (EMB) agar by streaking method. Biochemical test was carried out on the isolated bacteria using Bergey's Manual of Bacteriological Identification. Antibiogram of the isolated bacteria were determined by Kirby-Bauer disc diffusion method. Antimicrobial activity of clove extract was determined by well-in-agar method. Among the 30 sample of the adult female, only 20% of the urine sample were positive for the presence of *Escherichia coli* while 80% were devoid of *Escherichia coli*. In the case of urine sample from pregnant women, *Escherichia coli* was isolated from 45% of the sample while 55% of the sample showed no presence of *Escherichia coli*. The result of the antibiotic sensitivity showed that 14% of *Escherichia coli* were resistant to ofloxacin, 33% resistant to perfloxacin, 60%, resistant to augmentin, 74% resistant to nitrofurantoin, 73%, resistant to ceftazidime, 93% resistant to Nalidixic acid and 33% were resistant to gentamin. The antimicrobial activity of cloves produced zone inhibition ranged from 11.50±0.71 to 17.50±0.71mm of all the tested isolated. The antimicrobial potential of clove in the treatment of urinary tract infection by *Escherichia coli* was also shown in this study, hence could be applied in the treatment of urinary tract infections.

Keywords: *Antimicrobial, Ciprofloxacin, Cloves, Escherichia coli, Susceptible*

INTRODUCTION

Clove belongs to a tree Eugenia caryophyllata (*Syzygium aromaticum*) which is used as spices in most case. (Karkosh, 2012., Hussain *et al*., 2017). Clove oil has several active ingredients that describe its functionality. It primarily contains eugenol (48 - 89%), β-caryophyllene (5 - 22%) and eugenyl acetate (0.4 - 22%) (Hanif and Deepa, 2011; Mitta, *et al*., 2014; Cock and Cheesman, 2018). Additionally, it also possesses small quantity of α-humulene. Percentages of these functional components depend upon several factors such as variety of clove, part of plant, type of soil, plant genetics, climatic conditions and extraction methods (Khalil *et al*., 2017).

Recently, studies have been focused to extract the useful compounds from plants for applications in pharmaceutical, flavouring and food industries. Several plants have been investigated for their antioxidant and antimicrobial effects due to the presence of various functional ingredients in their essential oils. Similarly, essential oil obtained from clove buds also possesses several functional properties i.e., antibacterial, antifungal, antioxidant, antitumor, anti-inflammatory, insecticidal and flavour-imparting characteristics (Ishaq *et al*., 2019). (Cock and Cheesman, 2018) and headaches (Packyanathan and Prakasam, 2017).

Urinary tract infections (UTIs) are some of the most common bacterial infections, affecting 15million people each year worldwide. *Escherichia coli* is a major cause of urinary tract infection globally (Flores-Mireles, *et al*., 2015). Urinary tract infections are among the most prevailing infectious diseases with substantial financial burden on the society. The incidence of community-acquired urinary tract infection is highest in young women of recent; almost half of all women will experience at least one episode of urinary tract infection during their lifetime and nearly 1 in 3 women would have had at least one episode of urinary tract infection by the age of 24years. Urinary tract infection incidence increases with age for both male and female but predominantly in women. According to report, it is estimated that 10% of men and 20% of women over the year of 65years have asymptomatic bacteriuria (Vasudevan, 2014).

Improper treatment increases the rate of occurrence of the acute cystitis and phylonephritis from 15% to 45% and lack of proper treatment during pregnancy worsen the condition (Staerk *et al*., 2016). It has been estimated globally that UTIs result in as many as 8.3 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually. Although this infection affects both genders, women are the most vulnerable may be due to their anatomy and reproductive physiology. The prevalence also increases with advancing age, catheterization, sexual activity, menopause and prostate problems (Bassey *et al*., 2016).

The predominant organisms responsible for UTI are mostly the Enterobacteriaceae especially Eighty 80% of the infection is caused by *Escherichia coli*, however, other pathogens are 4 responsible for conferring UTI. Gram positive cocci are gaining worldwide importance and Staphylococcus species are one of the major pathogens belonging to the cocci group (Vasudevan, 2014). The study was aimed at determining the antimicrobial effect of clove on *Escherichia coli* isolated from the urine of female adults in Aluu community, Rivers State. Its Objectives were: (i) To isolate and identify *Escherichia coli* from the urine samples (ii) To determine the antimicrobial sensitivity effects of cloves on the *Escherichia coli* isolated.

MATERIALS AND METHODS

Sample Collection

Urine specimens were collected from 30 female adults and 20 pregnant women residing in Aluu community, Port Harcourt Rivers state. The female adults were instructed on the mode of collection of the urine sample. Subjects were adequately educated on precautions to prevent contamination of specimen. The specimens were collected into sterilized, wide necked, leak proof, plastic universal containers. The samples were transported aseptically to the laboratory for immediate analysis.

Microbiological analysis

Each specimen of urine was shaken properly to ensured homogeneity. The samples were culture on Eosin Methylene Blue (EMB) agar by streaking method (Nonzon *et al*., 2002). The cultured plates were incubated at 370C for 24hours. Plates with growth were indicated as significant bacteria while plates that showed growth only at 10-3 dilution were indicated as insignificant bacteria. To ascertain the count on the cultured plates, the colonies on the plates were counted in relation to their dilution factor and recorded before purification of culture. Based on cultural morphological characteristics (size, color, shape, texture, elevation, edge or margin type and opacity) on the cultured plates, the isolates were streaked on the nutrient agar to ensure the purity of particular isolates. Pure isolates of the different organisms were preserved on nutrient agar at 40C under good aseptic conditions for further analysis. Based on their morphological properties, colonial or growth characters, Gram staining reaction, microscopic examinations, physiological and biochemical characterization, the isolates were identified (Cheesbrough, 2010). The results were also compared with the characteristics described in Bergey's Manual of Determinative Bacteriology (1994).

Antibiogram of the Isolated Organisms

In vitro sensitivity pattern of the isolates was studied by Kirby-Bauer disc diffusion method using numbers of antibiotic discs. The inoculums were prepared by transferring colonies from the pure culture to broth (normal saline) and matched with 0.1 McFarland. The standardized inoculums were then applied onto Mueller Hinton (MHA) agar plate by soaking with sterile swap stick. The discs were then placed aseptically on the surface of the agar plate. The plates would be incubated at 37^{o}C for 24 hours for development of inhibition zone. The diameters of zone of inhibition were measured and the interpretation would be made according to Clinical and Laboratory Standards Institute chart.

Preparation of the Clove

Extract Clove was dried and grinded into fine powder. The plant material was dissolved in distilled water (2:15 w/v). The extract was filtered using Whatman's no 1 filter and stored in an air tight container in the refrigerator until use for antimicrobial activity.

Antimicrobial activity of clove extract

The antimicrobial activity of cloves against *Escherichia coli* was determined by using well-in-agar diffusion method. 0.5 MacFarland standard of *E. coli* was spread on newly prepared Mueller Hinton agar and three well was bore with the aid of a cup borer. 10 µL of the extract was dispensed into two of the well and the commercial antibiotic (nalidixic acid) was added to the third well which served as a positive control. The inoculated plates were placed on the plates followed by incubation at 37°C for 24 h and the zone of inhibitions measured for the of the isolates.

RESULT AND DISCUSSIONS

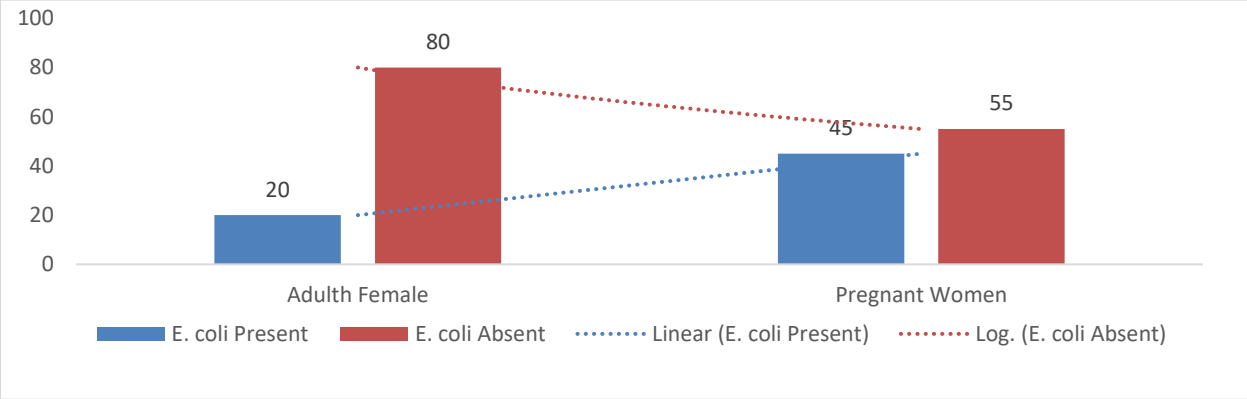

Figure 1: Percentage Prevalence of *E. coli* in Urine Samples

Table 1: Antibiotic Sensitivity of the Isolates

Isolate code	Microorganisms	OFL	PEF	AUG	NIT	CPR	CAZ	CRX	NA	GEN	CXM	%R
AF1	*Escherichia coli*	S	R	S	R	S	R	R	R	S	R	60
AF2	*Escherichia coli*	S	S	S	R	S	R	R	R	S	R	40
AF3	*Escherichia coli*	S	I	S	I	S	R	R	R	S	R	40
AF4	*Escherichia coli*	S	R	R	R	S	R	S	I	R	R	60
AF5	*Escherichia coli*	S	R	R	R	S	S	R	I	S	R	50
AF6	*Escherichia coli*	S	I	S	S	S	R	R	R	S	R	40
PW1	*Escherichia coli*	S	S	S	S	S	R	R	R	S	R	40
PW2	*Escherichia coli*	R	R	R	R	S	R	R	R	R	R	90
PW3	*Escherichia coli*	S	I	S	R	S	R	R	R	R	R	60
PW4	*Escherichia coli*	S	S	R	R	S	S	R	R	S	R	50
PW5	*Escherichia coli*	S	S	R	R	S	S	R	R	S	R	50
PW6	*Escherichia coli*	S	S	R	R	S	R	R	R	R	R	70
PW7	*Escherichia coli*	S	R	R	S	S	I	R	S	S	R	40
PW8	*Escherichia coli*	R	I	R	R	S	R	R	S	S	R	50
PW9	*Escherichia coli*	S	S	R	R	R	R	R	R	R	R	80
%R		13	33	60	73	7	73	93	73	33	100	

KEY:

OFL = Oflaxacin; PEF = Perfloxacin; AUG = Augmentin; NIT = Nitrofurantoin; CPR = Ciprofloxacin; CAZ = Ceftazidine; CRX = Co-trimoxazole; NA = Nalidixic acid; GEN = Gentamicin; CXM= Cefluroxime % R = Percentage Resistance

Table 2: Susceptibility Pattern of Bacteria Isolates to Clove Extracts (mg/ml)

Isolate code	Isolates	Zone of Inhibition
AF1	*Escherichia coli*	9.50±2.12
AF2	*Escherichia coli*	10.40±3.22
AF3	*Escherichia coli*	11.50±0.71
AF4	*Escherichia coli*	12.50±0.71
AF5	*Escherichia coli*	12.50±0.71
AF6	*Escherichia coli*	12.32±0.42
PW1	*Escherichia coli*	17.50±0.71
PW2	*Escherichia coli*	11.50±0.71
PW3	*Escherichia coli*	13.00±1.41
PW4	*Escherichia coli*	12.00±1.31
PW5	*Escherichia coli*	14.50±0.24
PW6	*Escherichia coli*	16.40±0.15
PW7	*Escherichia coli*	14.30±0.26
PW8	*Escherichia coli*	12.50±0.64
PW9	*Escherichia coli*	12.40±0.15

Alarmingly, uropathogens that cause urinary tract infections are becoming more resistant to antibiotics on a global scale. According to this study, *Escherichia coli* is among the primary cause of urinary tract infections. In this study, 50 urine samples from adult and pregnant females were analyzed; however, 15 (30%) of the samples tested positive for an *Escherichia coli*-caused urinary tract infection, while the remaining 70% tested negative. This might be the result of the participants' improper use of antibiotics (Ojo and Anibijuwon, 2010).

According to this research, 45% of the pregnant women whose samples were examined had an *Escherichia coli* infection. This may be due to pregnant women's higher susceptibility than adult females (20%) to urinary tract infections caused by *Escherichia coli* (Belete and Saravanan, 2020). According to Vasudevan (2014), incorrect diagnosis during pregnancy can lead to urinary tract infection, which increases the scope of infection and puts pregnant women at risk for life-threatening problems. This is consistent with research by Kadhim and Al-Hilali (2018), which found that 47 urinary tract infections were more common among pregnant women.

The majority of antibiotics in this study were also found to be ineffective in getting rid of *Escherichia coli*, which is the urinary tract infection's causative agent. On the other hand, the antibiotics oflaxacin, ciprofloxacin, and gentamin demonstrated greater efficacy in getting rid of *Escherichia coli*. This result is similar to other studies (Olabimtan *et al*., 2018; Oguntunbi *et al*., 2021). Oluwafemi *et al*., 2018 research study revealed that the causative organism is sensitive to nitrofurantoin and ciprofloxacin and ofloxacin but resistance to cotrimoxazole and tetracycline.

The overuse of antibiotics can be blamed for the growing bacterial resistance to urinary tract infection agents. 67%, 87%, and 93% of the isolates in this investigation were found to be sensitive to gentamicin, ofloxacin, and ciprofloxacin, in that order. Previous studies have also suggested that ciprofloxacin's effectiveness may stem from its broad-spectrum actions, bactericidal effects on organisms in both resting and replicating phases, and capacity to interfere with DNA processes, ultimately resulting in bacterial death. The way gentamycin worked was likewise comparable (Ojo and Anibijuwon, 2010). Since ceftazidime, augmentin, and nalidixic acid are the most often used antibiotics for treating infections, the high resistance of uropathogens to these drugs that was observed in this study is similar to that reported by Alanazi *et al*. (2018). This poses a serious threat to public health.

With different zones of inhibition, this investigation also demonstrated the clove extract's efficacy against *Escherichia coli*. The zone of inhibition that cloves recorded in this investigation, which ranged from 11.50±0.71 to 17.50±0.71 mm, is comparable to that reported in the study conducted by Ishaq *et al*. (2019) against a variety of harmful bacteria. Past research has demonstrated that clove extracts have strong antibacterial properties against a variety of bacteria, including *Salmonella typhi, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis* and *Escherichia coli*. Cook and Cheesman (2018), demonstrated clove extracts effective against five (5) bacterial strains. The active ingredients in clove extract, in particular eugenol, harm bacterial membranes and cause intracellular materials to leak out of the cells (Ishaq *et al*., 2019). The judicious use of antibiotics, which causes uropathogens to become more resistant to various antibiotics, can be decreased by using efficient natural organic compounds like cloves (Alexandra *et al*., 2013). It is essential that right dosage be administered. Cook and Cheesman, 2018 reported LD50 of 21.7±0.8 mg/ml.

CONCLUSION

The study's findings demonstrated the comparatively high incidence of *Escherichia coli* in female urinary tract

infections, particularly in expectant mothers. Since many of the isolates were found to be resistant to several of the antibiotics under investigation, the study demonstrated the prevalence of antibiotic resistance in *Escherichia coli*. This study also demonstrated the antibacterial capability of clove in treating urinary tract infections caused by *Escherichia coli*, suggesting that it may be used to treat urinary tract infections.

CONFLICTING INTEREST

All authors have read through the work. There is no conflict of interest.

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