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EFFECTS OF CRUDE ALKALOID EXTRACTED FROM *OCIMUM GRATISSIMUM* ON THE ACTIVITY OF CIPROFLOXACIN AGAINST *SALMONELLA ENTERICA* SEROVAR TYPHI

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ABSTRACT
Salmonella infection has been a major food-borne infection in Nigeria, and proliferations of multi-drug resistant strains of Salmonella possess a threat to public health. This work was carried out to investigate the effect of crude alkaloid extracted from *Ocimum gratissimum* leaf on the activity of ciprofloxacin against *Salmonella enterica* serovar Typhi. An egg white was aseptically collected from raw egg and plated on Salmonella Shigella agar (SSA) using pour plating method and incubated at 37°C for 24 h. Crude alkaloid was extracted from *Ocimum gratissimum* using solvent extraction method. Agar-well diffusion technique was used to evaluate the antibacterial activity of the alkaloid and in combination with ciprofloxacin against *Salmonella enterica* serovarTyphi. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using double-fold serial dilution technique at concentrations 25 mg/ml to 400 mg/ml. The results revealed that the crude alkaloid (16.70 mm) and Ciprofloxacin (23.33 mm) showed a pronounced inhibitory effect on the test organism. An antagonistic activity existed between the alkaloid and ciprofloxacin as zones of inhibition decreased to 27.33 mm. The results of the MICs and MBCs showed the inhibitory substances had significant inhibitory and cidal activities. The research further revealed that the crude alkaloid possesses antibacterial properties, so single dose of the crude alkaloid or the ciprofloxacin should be administered separately when managing *Salmonella* infections. Synergistic effects existed between the crude alkaloid in an increased concentrations (100, 150, 200,250,300 and 350mg/ml) with ciprofloxacin in a decreased concentrations (350,300,250,200,150 and 100mg/ml), of which equal volumes of the test substances at 200mg/ml each proved to be most effective against the test organism. The test substances at concentrations ≤ 50 mg/ml showed additive effects. This research has shown that the combined use of crude alkaloid and ciprofloxacin showed remarkable synergic effect against *Salmonella enterica* serovar Typhi, of which the combination of equal volume from 200 mg/ml of each substanceproved to be more effective.
Key words: *Salmonella enterica* serovar Typhi, crude alkaloid, *Ocimum gratissimum,* ciprofloxacin

INTRODUCTION

Salmonella enterica serovar Typhi being an obligate parasite has few known natural reservoir outside humans. Little is known about the historical emergence of human *Salmonella enterica* serovar Typhi infections. However it is thought to have caused the death of many famous figures such as British author and poet Rudyard Kipling, the inventor of the airplane, Wilbur Wright, and the Greek empire's Alexander the Great. The earliest epidemic occurred in Jamestown, VA where it was thought that 6,000 people died due to *Salmonella enterica* serovar Typhi infections in the early 17th Century according some group of pathologists (Ficker *et al.*2005).

This disease is rare in the United States and developed nations, but always poses the risk of emergence. *S.serovar* Typhi has been a major human pathogen for thousands of years, thriving in conditions of poor sanitation, crowding and social chaos. The name *Salmonella enterica* serovar Typhi was derived from the ancient Greek word typhos, an ethereal smoke or cloud that was believed to cause disease and madness. *Salmonella enterica* serova rTyphi has a combination of characteristics that makes it an effective pathogen. This species contain an endotoxin typical of Gram negative organisms, as well as the VI antigen which is thought to increase its virulence. It also produces and secretes a protein called invasin that allows non-phagocytic cells to take up the bacterium, where it is able to live intracellular. It is also able to inhibit the oxidative burst of leukocytes, making innate immune response ineffective. (Maji *et al.*,2005)

The encounter of human to *Salmonella enterica* serovar Typhi is made via fecal-oral route by ingestion of

contaminated food and water, the major problem encountered from this bacteria within the clinical setting is its multiple resistant strains and these strains of the bacteria usually emanate from abuse of several antibiotics thus making the treatment of the pathogen medically challenging, since antibiotics sensitivity test is usually not conducted before prescription of drugs by the medical personnel which can result to partial or ineffective treatment of salmonella infections (Maji *et* al., 2005). For so many centuries some medicinal herbs have been exploited in treatment of enteric diseases which have proven active and efficient against the bacteria (Sofowora, 1993).

From my enquiries I found out that one of the indigenous herbs which have proven a reliable means of treating salmonellosis, called *Ocimum gratissimum* in Uli village within the environs of Anambra State University which is locally referred to as scent leaf, has only been exploited through aqueous extraction and ethanol extraction of the herbs but little or no work has been done and published using the alkaloid extract from*Ocimum gratissimum* on *Salmonella enterica* serovar Typhi and possible synergistic,antagonistic or additive effect when administered to a patient alongside a conventional antibiotics, that became the driving motive that lead to my research work.

MATERIALS AND METHODS

Sample collection: The fresh leaves of *Ocimum gratissimum* was collected from Umuoma village Uli, Ihiala Local Government Area, Anambra State and authenticated appropriately.

Preparation of samples for Extraction: The leaves of *Ocimum gratissimum* were picked or plucked off the stem and dried under shade at room temperature for 14 days. The dried samples were pulverized using electric grinder, weighed and kept ready for extraction of active ingredients (Nwobu *et al.*2010).

Extraction of Alkaloid Crude Extract: This was carried out using the method of Chukwura and Iheukwumere (2013), 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the extract and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is diluted with the solvents.

Preparation of Test Sample: In this study, concentration of 400 mg / ml of the crude alkaloid was used to screen for the antimicrobial activity. This was carried out using the modified method of (Iheukwumere and Umedum, 2013). Here, 0.5g of the crude alkaloid and ciprofloxacin was dissolved in phosphate buffer saline (PBS).

Isolation, characterization and identification of isolated Organism: The test organism was isolated from egg. The egg was perforated with syringe through the air space to collect the egg white while the egg mixture was also taken and poured into the beaker, one millimeter of the sample was aseptically collected and plated on sabouraud dextrose agar at 35±2°C for 48 h. the colonies generated were sub cultured, characterized and identified using colony description, Gram staining and biochemical reactions.

Sensitivity Testing Using Agar-Welled Diffusion Method: Each labeled plate was uniformly inoculated with the test organism using pour plating method. A sterile cork borer of 5 mm diameter was used to make wells on the medium. One tenth milliliter (0.1 ml) of various concentrations of the crude alkaloid was dropped into each labeled well, and then incubated at 37^{o}C for 24 hours. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation. The same procedure was repeated using ciprofloxacin and combination of equal volumes of ciprofloxacin and crude alkaloid at different concentrations (Chukwura and Iheukwumere, 2013).

Determination of Minimum Inhibitory Concentration (MIC): This was carried out using the modified method of (Iheukwumere and Umedum, 2013). Here, various concentrations of the test substances were obtained using double-fold serial dilution. Each dilution was assayed against the test organism using tube dilution method. One milliliter of the test organism was added into each dilution and incubated at 37^{o}C for 24 h. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. This was determined and recorded.(Iheukwumere and Umedum, 2013).

Determination of Minimum Bacterial Concentration (MBC):This was carried out using the modified method of (Iheukwumere and Umedum, 2013).Here, equal volumes of various concentrations of those tubes that did not show any visible growth for MIC were sub cultured on sterile poured plate and incubated at 37^{o}C for 24 h. The lowest concentration of the extract that showed no visible growth is the MBC/ MLD (Iheukwumere and Umedum, 2013).

RESULTS

The test isolate was characterized and identified using colony description (colorless and dark centered in salmonella- shigella agar, entire and raised), Gram reaction (Gram negative rod), biochemical reactions (catalase +, hydrogen sulphide +, citrate - and ability to ferment glucose, maltose, sorbitol, xylitol and inositol),

morphological characteristics (motile) and positive result of slide agglutination test using antisera from infected human. The study revealed that the crude alkaloid and ciprofloxacin showed pronounced activity against the tested organism, of which the activity of ciprofloxacin was significantly ($P<0.05$) higher (Table 1). The combination of the crude alkaloid extracted from *Ocimum gratissmum and* ciprofloxacin at different concentrations showed synergistic effects. It was also observed that there were significantly ($P< 0.05$) increased in the activity of the crude alkaloid plus ciprofloxacin of which the activity at 400 mg/ml (crude alkaloid) and 200 mg (ciprofloxacin) proved to be most effective. Additive effect was observed at very low concentration of the ciprofloxacin and crude alkaloid (400 mg/ml). The MIC and MBC study revealed that ciprofloxacin and alkaloid combined at the most effective dose showed the most pronounced activity (Table 2), as the MIC and MBC values of ciprofloxacin were greatly reduced.

Table 1: Antibacterial activity of test substances using 5 mm cork borer

C A	CPX(mg/ml)	DZ (mm)	I
400	-	16.70±0.48	Effective
-	400	23.33±0.81	Effective
50	350	23.66±0.41	Additive
100	300	37.20±0.58	Synergistic
150	250	34.77±1.21	Synergistic
200	200	42.66± 0.81	Synergistic
250	150	33.33±1.33	Synergistic
300	100	28.50±0.58	Synergistic
350	50	23.77±0.41	Additive

CA = Crude alkaloid, CPX = Ciprofloxacin, DZ = Diameter zone, I = Inference

Table 2: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration(MBC) of the test substances.

Test substance	MIC(mg/ml)	MBC(mg/ml)
Alkaloid	400	-
CPX	100	200
AK (200mg/ml)+ CPX(200mg/ml)	25	100

AK = Alkaloid, CPX = Ciprofloxacin

DISCUSSION

In the present study the combination of crude alkaloid and ciprofloxacin at different concentration have shown remarkable synergistic effects against *Samonella enterica* serovar Typhi. Ciprofloxacin is a synthetic chemotherapeutic antibiotic of fluoroquinolone resistance is related to efflux pump that decrease the intracellular quinone concentration (Robert *et al*., 2014). Alkaloids work by intercalating into the cell wall or DNA of the organism; using ciprofloxacin in combination with crude alkaloid shows synergistic effect, most at 400 mg/ml (crude alkaloid) plus 200 mg/ml (CPX). According to Robert *et al*. (2014), alkaloid can work in synergism with ciprofloxacin enhancing its antibacterial activity. Similar findings was reported by many researchers (Chang *et al*., 2007; Chukwura and Iheukwumere, 2013; Ibrahim *et al*., 2014; Anabela *et al*., 2016). It was also observed that addition of 400 mg/ml of alkaloid to 200 mg of ciprofloxacin in equal volumes markedly reduced the MICs and MBCs of ciprofloxacin against the tested organism. This high synergism rate shows the need for more studies concerning the molecular basis of these interactions for better understanding of the pharmacological agents. Also, the reduced concentration of ciprofloxacin is expected to avoid the severity of administering this antibiotic. Therefore, proper concentration of biocide should be carefully chosen. Reducing development of resistance, providing broad coverage in poly microbial infection and consequently reducing the side effects and toxicity are some of the advantages of combined antimicrobial therapy (Robert *et al*., 2014). Therefore alkaloid can be used as adjunct to boast the efficacy of ciprofloxacin and also to reduce its side effects and toxicity.

CONCLUSION

The combined use of crude alkaloid and ciprofloxacin has shown remarkable synergistic effect against *Salmonella enterica* serovar Typhi at different concentrations of which crude alkaloid (200 mg/ml) and ciprofloxacin (200 mg/ml) proved to be most effective. Therefore, appropriate concentrations of alkaloid in combination with ciprofloxacin could be used as an alternative medicine for infections cause by the organism.

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