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Synergistic Effect of Xylopia Aethiopica Seed Extract and Ciprofloxacin on Salmonella Enterica Serovar Typhi

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Abstract
This study was carried out to evaluate the synergistic effect of *Xylopia aethiopica* seed extracts and Ciprofloxacin on *Salmonella enterica* serover Typhi. Egg white portion of raw egg was aseptically collected with sterile syringe and was plated on Salmonella Shigella agar (SSA), incubated at 37^0C for 24 h. The test organism was characterized and identified using their colony descriptions, morphology and biochemical characteristics. The phytochemical constituent of the seed extract of *Xylopia aethiopica* were determined quantitatively using spectophotometric method. The antibacterial activity of the seed extracts was carried out using agar-well diffusion method. Tube dilution method was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using double-fold serial dilutions at concentrations 25 mg/ml to 400 mg/ml. The synergistic activity was carried out by exposing the test organism against 0.1ml of equal volume of aqueous seed extract of *Xylopia aethiopica* and Ciprofloxacin (EEXA + CPX), and ethanolic seed extract of *Xylopia aethiopica* and Ciprofloxacin (AEXA + CPX) at the same concentration (400 mg/ml). The phytochemical analysis of *Xylopia aethiopica* seed extract revealed the presence of alkaloid, saponins, flavonoids, steroids, cardiac glycosides, phenolics and tannins. Ethanolic extract of *Xylopia aethiopica* showed more activity (15.50 mm) than the aqueous extract (6.50 mm) and their activities differed significantly (P<.0.05) from that of the Ciprofloxacin (18.00 mm).There wassynergistic effects on EEXA + CPX (24.50mm) and AEXA + CPX (21.00mm). The MICs and MBCs values of the extracts and their combinations revealed significantly the inhibitory and cidal activities. The study suggest that the combination of the seed extracts of *Xylopia aethiopica* and Ciprofloxacin could perform better in management of typhoid fever than monotherapy, and ethanolic extract combined with CPX proved to be most effective.

INTRODUCTION

Salmonellosis is a contagious endemic bacterial infection associated with substantial morbidity and mortality rates in India, Southeast Asia, Africa and South America. Typhoid fever is caused by *Salmonella enterica serovar* Typhi, a rod-shaped, gram negative bacteria belonging to the family of Enterobacteriaceae. *Salmonella* species especially *S. enterica* serovar Typhi causes a significant threat to public health by causing salmonellosis. (Febrega and Vila., 2013).*Salmonella* infection is typically by ingestion (faecal oral contamination) and concentrates in areas that are not hygienic and do not possess adequate waste management and safe drinking water supplies (Febrega and Vila, 2013). Salmonellosis is associated with 10-20% and 20-40% of fatal cases of diarrhea worldwide .Though, children are at greater risk of getting the disease even persons that has a close contact with infected patients, immune system weakened by medication and drinking water or eating food contaminated by *S. enterica serovar*Typhi. Without proper care, salmonellosis can become life threatening (Santos *et al.* 2001).

Over the years, the vigorous use of oral fluids and electrolytes therapy in developing countries has contributed significantly to reductions in mortality from typhoid fever (Santos *et al.* 2001).In contrast this invention provides little benefit to enteropathogens such as *Salmonella.* Over the past years *Salmonella* has demonstrated extraordinary prowess in acquiring plasmid–encoded resistance to the antimicrobial drugs like ampicillin, chloramphenicol, trimethoprim– sulfamethazole, streptomycin and ciprofloxacin that previously constituted first–line therapy (Strokes *et al*. 1996). Innovative strategies including development of vaccines against the most common serotype, show great promise for the prevention of typhoid fever(Patil and Shethgar,2010)

The practice of using of natural sources as remedy or alternative medicine for the treatment of typhoid has been proven effective in recent years. *Xylopia aethiopica* which belongs to the family Annonaceae (custard apple family) is native to West Africa and a known home remedy for treating typhoid fever. It is known to possess antimicrobial activities which are traced to its spicery and medicinal purposes. Various works have been done by researchers using *Xylopia aethiopica* extracts like "Phytochemical and antimicrobial studies of extract of the fruits of *Xylopia aethiopica*" ,by John-derwole (2007), and Burkhill (2000) did a work on the effect of *Xylopia aethiopica* on skin infection. In view of the clinical burden of drug resistance of *Salmonella enterica* serovar Typhi to most antibiotics and being a public threat to human health, this research work was undertaken to know

the synergistic effect of *Xylopia aethiopica* seed extract and ciprofloxacin on *Salmonella enterica* serovar Typhi.

MATERIALS AND METHODS

Sample and Collection: The fresh seeds of *Xylopia* aethiopica were collected from Umuobia village Umuoji, Idemili North Local Government Area, Anambra State, and athenticated appropriately.

Preparation of Sample for Extraction: The seeds of *Xylopia aethiopica* were plucked off and dried under shade of room temperature for 14days. The dried samples were pulverized using electric grinder, weighed and kept ready for extraction of active ingredients (Nwobu *et al.* 2010).

Extraction Procedure: A 20 g portion of the sample was extracted by maceration in 200ml of ethanol and water respectively for 3days. The resulting extracts were subsequently filtered using What man N0.1 filter paper. The ethanolic and aqueous extracts will be evaporated to dryness at room temperature in a steady air current (Nwobu *et al.*, 2010).

Preparation of Test Sample: In this study, concentration of 400 mg/ml of the extracts was used to screen for the antimicrobial activity. This was done by using the modified method of Iheukwumere and Umedum,(2013). Here 2.5 g of the extract was dissolved in 6.25 ml each of the peptone water.

Isolation and Identification of Test Organism: The test organism used for this work was collected from raw eggs from various shops from Umuoma village Uli town in Ihiala Local Government Area. The samples were collected aseptically using sterile syringe and were plated on Salmonella-Shigella agar and incubated at 37^0C for 48 h. The organism obtained ascetically plated on nutrient agar plate and incubated at 37^0C for 24 h. The pure culture of the organism was identified using colony description, morphology, biochemical reactions and slide agglutination test (Iheukwumere and Umedum,2013)

Maintenance of Test Organism: The isolated organism was used for the antibacterial sensitivity testing. Prior to the test, the organisms were sub-cultured on nutrient agar at 37^0C for 24 h. Then the 24h culture was transferred into nutrient broth and incubated at 37^0C for 24 h. (Iheukwumere and Umedum, 2013)

Sensitivity Testing Using Agar Well Diffusion Method: This was carried out using the modified method of (Iheukwumere and Umedum, 2013). 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 millitre (0.1 ml) of various concentrations of the extracts were dropped into each labeled well, and then incubated at 37^0C for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation. This same procedure was repeated for CPX, combination of CPX and XAS with equal volume at the same concentration.

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 extracts 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^oC for 24 h. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. This was determined and recorded.

Determination of Minimum Bacterial Concentration: This was determined 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 pour plate and incubated at 37^0C for 24 h. The lowest concentration of the extracts that showed no visible growth is the MBC.

RESULT

The quantitative phytochemical analysis of the seeds of *xylopia aethiopica* were shown in table1.The result revealed the presence of flavonoids phenolics, tannins, alkaloids, saponins, cardiac glycosides and steroids of *Xylopia aethiopica*. These phytochemical constituents may be responsible for the activity of the seed extracts of *Xylopia aethiopica.* The characteristic and identities of the test isolates were presented in Table 2. The isolated organism was *Salmonella enterica* Typhi. These organisms were characterized and identified using its colonial description, Gram staining reaction and biochemical reactions.The diameter zones of inhibitions of *Xylopia aethiopica* seed extracts, ciprofloxacin and extracts plus ciprofloxacin against *Salmonella enterica* serovar Typhi were presented in Table 3.The results revealed that significant ($P<0.05$) inhibitions were seen when the extracts were combined in equal proportion with ciprofloxacin, and ciprofloxacin combined with ethanolic extract significantly ($P<0.05$) inhibited the organism better than ciprofloxacin combined with aqueous extract. The results of the MIC and MBC showed pronounced activities of the inhibitory substances.

Table 1: Quantitative photochemical constituents of *Xylopiaaethiopica*seed extracts

Phytochemical	Amount (%)
Flavonoids	0.32
Phenolics	1.24
Tannins	0.58
Alkaloids	1.84
Saponins	0.26
Cardiac glycosides	0.33
Steroids	0.07

Table 2: Characteristic and identity of the test organism

Parameter	*Salmonella entericaTyphi*
Appearance on agar plate	Red colony on SSA that darkened at the centre
Gram reaction	-
Morphology	Rod shaped
Catalase	+
Motility	+
H_2S	+
Citrate	+
SAT	+

Key + = positive,- =negative SAT = Slide Agglutination Test , SSA =*Salmonella-Shigella* Agar

Table 3: Diameter zones (mm) of inhibition of *Xylopica aethiopica* seed extract against the test isolate using 5 mm cork borer.

Extract (400mg/ml)	*Salmonella enterica ser.*Typhi ($X \pm \overline{SD}$) MM
EEXA	15.50 ±4.56
AEXA	6.50 ± 4.56
CPX	18.00 ± 4.56
CPX + EEXA	24.50 ± 3.75
CPX + AEXA	21.00 ± 3.75
Absolute Ethanol (0.1ml)	-
Distilled H_2O (0.1 ml)	-

Key: EEXA = Ethanol extract of *Xylopia aethiopica*, AEXA= Aqueous Extract of *Xylopia aethiopica*
CPA = Ciprofloxacin, CPX + EEXA = Ciprofloxacin and ethanol extract of *Xylopia aethiopica.*
CPX + AEXA = Ciprofloxacin and aqueous extract of *Xylopia aethiopica*., -= No effect / Nill

Table 4: The minimums inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Test substance	MIC (mg/ml)	MBC (mg/ml)
EEXA	200	400
AEXA	400	-
CPX	200	400
CPX+EEXA	100	200
CPX+AEXA	100	200

EEXA=Ethanol extract of *Xylopia aethiopica*, AEXA=Aqueous extract of *Xylopia aethiopica*
CPX=Ciprofloxacin, CPX+EEXA=Ciprofloxacin and Ethanol extract of *Xylopia aethiopica*
CPX+AEXA=Ciprofloxacin and Ethanol extract of *Xylopia aethiopica*

DISCUSSION

Medicinal plants extract have been identified throughout human history due to its ability to synthesizes a wide varieties of chemical compound that are used to perform important biological functions, and its defense against attack from predators such s insects, fungi and herbivorous animals (Summer *et al.*2000). Herbal medicines are as effective as conventional medicine, but have the same potential to cause harmful side effects. In this study, the synergistic effect of *Xylopia aethiopica* seed extract and ciprofloxacin on *Salmonella enterica serovar* Typhi isolated from raw eggs was evaluated .The data clearly revealed a pronounced activity of the extract against the test organism. The presence of these phytochemicals in the studied extract maybe responsible for the anti-bacterial activity exhibited by the seed extract of *Xylopia aethiopica*. Similar findings were made by different researcher (Patil and Shethgar, 2010; Iheukwumere *et al*., 2012; Iheukwumere and Umedum, 2013). Some

phytochemical work by intercalating with DNA of the organism (alkaloid) interfere with protein synthesis and disrupt cell membrane (eg saponin) while others interfere signal transduction pathway, metabolic processes, damage metabolic and cellular enzymes, disrupt proton motive force, electron flow, coagulation of cell component and modulation of gene expression (Kris-Etherthon *et al.,* 2002).

The ethanolic extract showed more activity against *S.enterica* serovar Typhi than the aqueous extract. This showed that active phytochemical constituent of the seed had more ability to dissolve in ethanol (organic solvent) than in water (in organic solvent). Similar conclusion was drawn by different researchers (Scalbert, 1991; Naibe *et al*.2008).Though aqueous extract produced higher amount of extract but exhibited relatively lower activity than the ethanolic extract which was obtained in lower quantity. This indicates that the amount of yield did not always influence the inhibition of microbial growth but the active ingredient found in the extract play the major role. Similar observation was made by Iheukwumere and Umedum (2013). They further highlighted that ethanol was able to extract more of the phytochemical constituents because ethanol is an organic and polar solvent, and most of the phytochemical constituents are organic nature.

The reduction in the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the combined test substances revealed the synergistic effect that existed between the test substances, which remarkably enhanced their bactericidal activities.This means that infections caused by *S. enterica* serovar Tyhpi could be managed effectively using the combination of X*ylopia aethiopica* seed extract and ciprofloxacin . Also, further research involving in vivo assays will be needed to establish the relationship between the MICs and MBCs obtained in this study and effective dosage that should be administered in ethnomedical practice.

CONCLUSION

The study has proven that the combination of X*ylopia aethiopica* seed extract and ciprofloxacxin showed remarkable synergistic effect against the test organism of which the ethanolic extract combined with ciprofloxacin proved more effective.

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