IN VITRO COMBINED ANTIBACTERIAL EFFECT OF TURMERIC (*Curcuma longa)* AND GINGER (*Zingiber officinale)* ON SOME PATHOGENIC ORGANISMS

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Abstract. The antibacterial effects *in vitro* of crude ethanol and aqueous extracts of Turmeric (*Curcuma longa)*, Ginger (*Zingiber officinale)* and Turmeric and Ginger combined were assayed against *E. coli, S. aureus, P. aeruginosa* and *S. enterica* Type typhi. The antibacterial activity was determined using the well diffusion method. Turmeric (*C. longa)* showed least antibacterial activity against the test organisms. The combined extracts of *C. longa* and *Z. officinale* exhibited higher antibacterial activity than *C. longa* alone. Ginger (*Z. officinale)* extracts were most effective. Ethanolic extracts were found to be more potent than the aqueous extracts. The plants' extracts were least active against *S. enterica* Type typhi and most active against *P. aeruginosa*. Chloramphenicol which served as the control had higher activity than the spices singly and combined. The Minimum Inhibitory Concentration (MIC) of the ethanolic extracts of plants was 50-150 mg/ml for *C. longa,* 50-100 mg/ml *Z. officinale* and 50-150 mg/ml in synergy. Results of this kind exhibit an interesting promise of designing a potentially active antibacterial combined agents of plant origin.

Keyword: Combined; Antibacterial effect; Turmeric; Ginger; Chloramphenicol; Pathogenic organisms.

INTRODUCTION

Medicinal plants have a long history of use and their uses are wide spread all over the world. According to the report of the World Health Organization, 80% of the world's population rely mainly on traditional therapies which involve the use of plant extracts or their bioactive substances [22]. Plants have potent bioactive substances that are harnessed in phytomedicine [27].

The increase in antibiotic resistant bacteria is largely due to the widespread use of antibiotics in medicine, in animal care, and in agriculture. The lack of new antibiotics to attack bacteria in different ways to circumvent the resistant genes poses a great challenge. Decreasing efficiency and resistance of pathogens to antimicrobial drugs made the search of a new antimicrobial agent an important strategy for the establishment of alternative therapies in difficult handling infections [13].

The natural products are found to be more effective with least side effects as compared to commercial antibiotics, so for this reason plants are used as alternative remedy for treatment of various infections [25]. They are also less expensive, acceptance due to long history of use, and being renewable in nature [7]. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [26].

Ginger (*Zingiber officinale*) and Turmeric (*Curcuma longa*) are members of the family Zingiberaceae; a small family with more than 45 genera, and 800 species [12]. The two plants are used as addictives in foods. They are used as species, colorant, preservative and are known to posses medicinal values [28]. In Ayurveda, *Zingiber officinale, Curcuma longa* and *Curcuma amada* rhizomes are most commonly used due to medicinal values [24, 21].

Ginger is truly a world domestic remedy. Fresh ginger has been used for cold-induced diseases, nausea, asthma, cough, colic, heart palpitation and swelling. *In vitro* studies have shown that active constituents of ginger inhibit multiplication of colon bacteria. These bacteria ferment undigested carbohydrates causing flatulence, this can be counteracted with ginger [23]. Tumeric has been used topically on the skin for wounds, blistering diseases such as pemphigus and herpes zoster, for parasitic skin infections, and for acne. It has been used via oral administration for the common cold, liver diseases, urinary tract diseases, and as a blood purifier. For chronic rhinitis and coryza, it has been used via inhalation [8].

The present study was undertaken to analyze the antimicrobial potentials of *Curcuma longa* and *Zingiber officinale* extracts and their combined effect against four human pathogens which include *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella enterica* Type typhi.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh rhizomes of *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) were collected from the orchard of National Root Crops Research Institute, Umudike, Abia State, South–East of Nigeria. The authenticating of the plant materials was done in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State. It was ensured that the rhizomes were healthy and uninfected. The rhizomes were washed thoroughly under running tap water to eliminate dust and foreign particles after which they were cut aseptically into small pieces, air-dried and grounded into powdery form using sterile manual grinder. They

were then stored in a cool, dry place prior to extraction process

Ethanol and Aqueous Extraction Preparation

Ten grams of dried plant was extracted with 100 mL of 95% ethanol in conical flasks sealed with foil and allowed to stand for 72 hours they were filtered to obtain crude ethanolic extracts and stored at 4^0C when not in use. Same was done for aqueous [11].

Preparation of the extracts for Synergy Test

10ml of the ethanolic extracts of *Z. officinale* was added to 10ml of corresponding extract of *C. longa* to get 1:1 ratio. This was repeated for the aqueous extracts.

Collection of Test Organisms

The test organisms were collected from stock cultures from Research Institute Microbiology Laboratory Unit, Umuahia, Abia State, Nigeria. The isolates were subjected to Gram staining and other biochemical tests according to standard procedures and identified as *E. coli, S. aureus, P. aeruginosa* and *S. enterica* Type typhi.

Antibacterial Assay

Antibacterial activity of the plants'extracts were tested using the agar well diffusion method [16]. The prepared culture plates were inoculated with the different selected strains of test organisms using streak plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were dispensed into the well. The plates were incubated at 37ºC±2ºC for 24 hours. The plates were observed for the zone clearance around the wells. The concentrations (50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml) of the various plant extracts were tested against different bacterial pathogens. The plates prepared were left at room temperature for 10minutes allowing the diffusion of the extract into the agar. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. A standard control, Chloramphenicol was used in a separate plate for each of the test organisms and the diameter of the inhibition zone was measured and recorded. Antimicrobial assay was performed in triplicate with each bacterial strain.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of the extracts was determined by diluting to various concentrations according to the macro broth technique [14]. Standard inoculum of each organism to be tested was added to series of tubes of nutrient broth containing two fold dilution of the extract and incubated at 37^0C for 24hours. The MIC was read as the least concentration that inhibited the growth of the test organisms.

Statistical analysis

All determinations were carried out at least in triplicates. Data were expressed as mean ±SEM. The diameter means of the bacterial growth inhibition zones were separated by using SPSS package (version 17.0).

RESULTS

The aqueous, ethanolic and combined effect of *Curcuma longa* and *Zingiber officinale* showed varying degrees of antibacterial activity against the test organisms, *S. aureus, E. coli, P. aeruginosa* and *S. enterica* Type typhi. The results obtained in tables 1-6 showed that *P. aeruginosa* was more sensitive to the plant extracts than the other test organisms while the least susceptible was *S. enterica* Type typhi. It was also observed that the ethanolic extracts have higher extracting potency than the aqueous extracts that produced mild antibacterial activity against all the test microorganisms. Also the results showed that *Zingiber officinale* showed higher activity than *Curcuma longa* against the test organisms. The Minimum Inhibitory Concentration (MIC) of the ethanolic extracts of plants was 50-150mg/ml for *C. longa,* 50-100mg/ml *Z. officinale* and 50-150mg/ml in synergy (Figure 1).

Table 1. Aqueous extract of *Curcuma longa* against the test organisms

	Concentration of the extracts (mg/ml)				
Test organism	200	150	100	50	Chloramphenicol
S. aureus	15.50±0.28	-	-	-	26.66±0.35
E. coli	16.80±0.37	-	-	-	22.76±0.43
P. aeruginosa	19.50±0.30	15.33±0.33	-	-	29.92±0.15
S. enterica Type typhi	14.70±0.43	-	-	-	24.59±0.32

Key: - No Inhibition

Table 2. Ethanolic extract of *Curcuma longa* against the test organisms

	Concentration of the extracts (mg/ml)				
Test organism	200	150	100	50	Chloramphenicol
S. aureus	18.56±0.46	15.88±0.49	-	-	26.66±0.35
E. coli	21.33±0.70	18.40±0.32	14.40±0.45	-	22.76±0.43
P. aeruginosa	23.16±0.81	19.50±0.28	15.76±0.39	12.70±0.17	29.92±0.15
S. enterica Type typhi	16.93±0.17	16.10±0.43	-	-	24.59±0.32

Key: - No inhibition

Table 3. Aqueous extract of *Zingiber officinale* against the test organisms

Test organism	Concentration of the extracts (mg/ml) 200	150	100	50	Chloramphenicol
S. aureus	16.58±0.33	12.23±0.24	-	-	26.66±0.35
E. coli	18.97±0.43	15.61±0.36	-	-	22.76±0.43
P. aeruginosa	21.67±0.20	16.04±0.19	12.40±0.23	-	29.92±0.15
S. enterica Type typhi	16.11±0.14	12.30±0.17	-	-	24.59±0.32

Key: – No inhibition

Table 4. Ethanolic extract of *Zingiber officinale* against the test organisms

Test organism	Concentration of the extracts (mg/ml) 200	150	100	50	Chloramphenicol
S. aureus	21.07±0.11	19.36±0.20	16.53±0.17	-	26.66±0.35
E. coli	25.10 ±0.17	20.83±0.08	16.06±0.20	-	22.76±0.43
P. aeruginosa	26.26 ±0.18	22.60±0.21	19.31±0.17	17.16±0.16	29.92±0.15
S. enterica Type typhi	19.06 ±0.12	16.20±0.17	12.43±0.20	-	24.59±0.32

Key: - No inhibition

Table 5. Combined effect of aqueous extracts of *C. longa* and *Z. officinale* against the test organisms

Test organism	Concentration of the extracts (mg/ml) 200	150	100	50	Chloramphenicol
S. aureus	15.46±0.24	11.06±0.12	-	-	26.66±0.35
E. coli	18.33±0.12	16.40±0.30	-	-	22.76±0.43
P. aeruginosa	21.46±0.08	17. 73±0.14	-	-	29.92±0.15
S. enterica Type typhi	14.26±0.14	-	-	-	24.59±0.32

Key: - No inhibition

Table 6. Combined effect of ethanolic extracts of *C. longa* and *Z. officinale* against the test organisms

Test organism	Concentration of the extracts (mg/ml) 200	150	100	50	Chloramphenicol
S. aureus	20.53±0.27	19.76±0.03	14.51±0.14	-	26.66±0.35
E. coli	22.56±0.17	19.20±0.05	16.59±0.18	-	22.76±0.43
P. aeruginosa	26.73 ±0.12	20.71±0.10	17.57±0.15	14.98±0.16	29.92±0.15
S. enterica Type typhi	18.23 ±0.20	13.52±0.14	-	-	24.59±0.32

Key: - No inhibition
Values are expressed as Mean ± SEM, n= 3

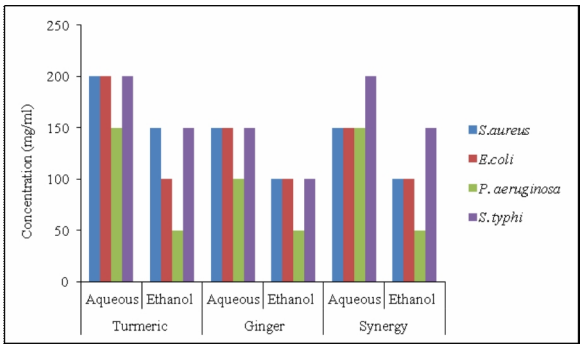

Figure 1. Minimum Inhibitory Concentration (MIC) mg/ml of the plant extracts

DISCUSSIONS

Recurring epidemics of drug resistant bacteria have necessitated the need to search for alternative antimicrobials. This research work evaluated the antibacterial activity of aqueous and ethanolic extract of *Curcuma longa* and *Zingiber officinale* and their combined effect and compared them with the antibiotic control on four test organisms, *S. aureus*, *E. coli, P. aeruginosa* and *S. enterica* Type typhi. The results showed that the isolates behaved differently in their sensitivity to the different extracts added to their growth medium.

Aqueous extract of *C. longa* inhibited all the test organisms only at 200mg/ml concentration excepting *P. aeruginosa* which was also sensitive at 150mg/ml with diameter zone of inhibition 10mm. Aqueous extracts of turmeric showed antioxidant and antimicrobial activity due to the presence of curcumin (5%), a polyphenolic compound [2]. Turmeric contains phenolic compounds called curcuminoids that possess all the bio-protective properties of turmeric. Crude turmeric extracts have both antioxidant and antimicrobial capacities so that turmeric could be a potent alternative to common antibiotics [5].

Ethanolic extract gave higher antibacterial activity than aqueous extract. This agrees with the findings of Mukhtar and Ghori [10] who reported that ethanolic extract of *C. Longa* gave a higher antibacterial activity on the test organisms than aqueous extract and Chandrana *et al*. [2] who reported that turmeric was effective against *E.coli* and *S. aureus*. The antimicrobial property of turmeric has been attributed to the presence of essential oil, an alkaloid, curcumin and other curcuminoids, turmeric oil, turmerol and veleric acid [3].

At concentrations 150 mg/ml–200mg/ml, the aqueous extract of *Z. officinale* inhibited the test organisms, *E. coli* 19.7mm, *S. aureus* 16.7mm and *S. enterica* Type typhi 16.4mm except *P. aeruginosa* which was further inhibited at 100mg/ml with zone of

inhibition of 12.8mm. However, this did not agree with the findings of Akintobi *et al.* [1] who reported that the water extract of ginger did not show any inhibitory effect against *E. coli* and *P. aeruginosa*. The test organisms were found to be less susceptible to the aqueous extract of *Z. officinale* than the ethanolic extract. All tested bacterial strains showed poor susceptibility to the ginger aqueous extract in a study by Gull *et al*. [6].

Ethanolic extract of *Zingiber officinale* had a higher inhibitory activity against all the test organisms than aqueous extract. It inhibited *P. aeruginosa* at all concentrations while *E. coli*, *S. aureus* and *S. enterica* Type typhi were inhibited at concentrations 100mg/ml-200mg/ml. This agrees with the study by Melvin *et al*. [9], it was found that the ginger extract exhibited maximum inhibitory effect against P. aeruginosa while the antimicrobial activity against *E. coli* was found to be moderate. Akintobi *et al*. [1] reported that the ethanol extract of *Zingiber officinale* had inhibitory effect on *S. enterica* Type typhi, *S. aureus* and *P. aeruginosa* and was ineffective against *E. coli*. They assumed the reason to be as a result of genetic differences between *Zingiber officinale* and microbial strains used in the study. Pathmaraj [20] reported the antimicrobial activity of ginger against *S. aureus.* Omoya and Akharaiyi [17] reported the inhibition of *E. coli* by ginger.

Comparatively, the *Z. officinale* extracts were more potent than the *C. longa* extracts. This agrees with the findings of Sayyad and Chaudhari [21] while Panpatil *et al*. [18] had a contrary finding that *C. longa* extracts were more potent than *Z. officinale* extracts.

When combined, the aqueous extract of the plant inhibited all the test organisms at concentrations 150mg/ml–200mg/ml except *Salmonella enterica* Type typhi which was only susceptible at 200mg/ml with diameter zone of inhibition 9.3mm. The combined effect water extract was higher than that of turmeric and almost the same with the aqueous extract of ginger. The combined effect of the ethanolic extract of both plants was found to be more active than the aqueous extract with *Salmonella enterica* Type typhi being less sensitive. The recorded activities of the *C. longa* and *Z. officinale* extracts were found to complement available reports. Okigbo *et al*. [25] reported that the extracts of three African tuberous plants, that is *Zingiber officinale, Curcuma longa*, and *Diocore abulbifera* contained tannins, phenols, saponins, alkaloids, flavonoid and steroids triterpenes and that these biological active chemical compounds were potent antimicrobials on three human pathogens – *E. coli* , *S. aureus* and *Candia albicans.*

The Minimum Inhibitory Concentration (MIC) of the plant extracts was higher in aqueous fractions of *C. longa* (150-200mg/ml), *Z. officinale* (100-150mg/ml) and in synergy (150-200mg/ml). Our present findings show that the ethanolic fractions of both plants have more potential as an antimicrobial agent than their aqueous fractions.

The differences in the antibacterial activity of the plant extracts might be attributed to the method of plant extraction [7]. Other reasons might be due to time of plant collection, herbal material nature, plant part and climate [19].

In comparism with the standard antibiotics (Chlormphenicol) which served as a control, produced higher diameter zone of inhibition than the aqueous and ethanolic extracts of both plant against all the test organism and when combined.

In addition, it was also observed that the higher the concentrations of the plant extracts, the higher the bacterial sensitivities as shown by the increased size of inhibition zones of the bacterial growth [4,15] shared the same technique of increasing the active antimicrobial substances in plant extracts to obtain better antimicrobial effects in their study.

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