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Comparative Study of Antibacterial Effects of *Curcuma longa* Linn. and *Zingiber officinale* Rosc. Rhizomes

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Authors' contributions

This work was carried out in collaboration between all authors. Author LAA designed the experiment, carried out laboratory work and contributed to the protocol (writing of the manuscript). Author TOS provided technical assistance and contributed to the protocol. Authors FAE and AAA carried out laboratory work and contributed to the protocol. Author SAA contributed to the experimental design and the protocol. Author RMS supervised the work, performed the statistical analysis and contributed to the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate and compare the antibacterial effect of *Curcuma longa* with *Zingiber officinale* rhizome ethanolic extracts against five bacterial species which include three gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*) and two gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*).
Study Design: Activity directed antibacterial effect of *C. longa* and *Z. officinale* using *in vitro* methods.
Place and Duration of Study: Medicinal Plants Section, Bioresources Development Centre, Ogbomoso, Nigeria between March and September, 2016.

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Methodology: The ethanolic extract was prepared by separately weighing 20, 40 and 60 g of powdered *C. longa* and *Z. officinale* into 100 mL of absolute ethanol. The antibiotic sensitivity test was carried out using a sensitivity disc impregnated with various antibiotics including Augmentin (AUG), Ampicillin (AMP), Ciprofloxacin (CIP), Ceptriaxome (CEP) and Ofloxacin (OFL).
Results: 40 and 60 g of *C. longa* and *Z. officinale* extracts significantly ($P < 0.05$) inhibited all the isolates (more effect was on *S. pyogenes*, *S. aureus*, *E. coli*, *P. mirabilis* followed by *K. pneumoniae*) when compared with 20 g. Also, 40 g of *Z. officinale* extract significantly ($P< 0.05$) inhibited the growth of *S. pyogenes* when compared with *C. longa* while 60 g of *C. longa* significantly ($P < 0.05$) inhibited the growth of *P. mirabilis* and *K. pneumonia* when compared with *Z. officinale*. The minimum inhibitory concentration of both extracts that inhibited *E. coli* and *P. mirabilis* was 1.3 g. CIP followed by CEP and OFL were active on all the bacteria used for this study. AUG was slightly active only on *K. pneumonia* while AMP was not active on any of the test organisms.
Conclusion: The result of this study showed that all the gram-positive bacteria used in this study were more sensitive to both *C. longa* and *Z. officinale* rhizomes ethanolic extracts as compared to the gram-negative bacteria.

Keywords: Curcuma longa; Zingiber officinale; rhizomes; antibacteria; antibiotics.

1. INTRODUCTION

Antimicrobial is a general term given to medicines that kill or slow down the growth of microbes [1]. The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have become a burning predicament [2]. There is also report that microbes have evolved resistance to practically all drugs [3]. Thus, there is an urgent need to find alternative chemotherapeutic drugs in diseases treatment particularly those of plants origin which are easily available and have considerably less side effects [4]. Also, the use of higher plants and their extracts for treating infectious diseases has long been practiced in many parts of the world [5].

Zingiber officinale Rosc. Commonly known as ginger, belongs to the family Zingiberaceae [6]. *Z. officinale* is a perennial annual plant cultivated for its rhizome. It is used as a raw material in the production of beverages, perfumes and medicines. The stems are erect, oblique, round, annual, 2-3 feet in height and invested by the smooth sheaths of the leaves. The plant has strong aromatic and medicinal properties and is characterized by its tuberous or non-tuberous rhizomes [7]. *Z. officinale* is a medicinal plant that has been widely used all over the world since ancient times for the treatment of many ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases [8]. It has been reported that ginger has direct anti-microbial activity and thus can be used in the treatment of bacterial infections [9] and also prevent the growth of many bacteria and fungi *in vitro* [10]. Recently, there has been a considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods [11]. The extract of the rhizome of ginger also has pronounced inhibitory activities against *Candida albicans* that causes candidiasis [12]. Ginger compounds are active against diarrhea which is the leading cause of infant death in developing countries. Zingerone is likely to be the active constituent against enterotoxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhea [11], [13]. It has also been reported that ginger extract has antimicrobial activity against food-borne pathogenic bacteria like *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio cholerae*, *Klebsiella* spp. and *Salmonella* spp. There has been a synergistic effect of ethanol extract of ginger and garlic against *Bacillus* spp. and *Staphylococcus aureus* and also an antimicrobial activity of ethanol extract of ginger, lime and garlic against broad range of bacteria including *Bacillus* spp., *Staphylococcus aureus, Escherichia coli*, and *Salmonella* spp [14].

Curcuma longa which is known commonly as turmeric also belongs to Zingiberaceae family [15]. It is widely used as spice, colouring agent and is also known for its medicinal values [16].

The aim of this study is to investigate and compare the antibacterial effect of *Zingiber officinale* and *Curcuma longa* rhizome ethanolic extracts on some isolated human pathogenic bacteria.

2. MATERIALS AND METHODS

2.1 Plant Material

Rhizomes of *Curcuma longa* were obtained from Medicinal Plants Section, Bioresources Development Centre, Ogbomoso, Nigeria. The plants were identified and authenticated at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. A specimen with voucher number: IFE-17578 and IFE-17577 for *Curcuma longa* and *Zingiber officinale* respectively were deposited at Ife Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.2 Collection of Test Organisms

The test organisms used for this study include; three gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis*) and two gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and were collected from the department of Medical Microbiology and Parasitology Laboratory, Bowen University Teaching Hospital, Ogbomosho, Osun State, Nigeria. The test organisms were resuscitated on agar slants and stored in the refrigerator at 4℃.

2.3 Preparation of *Curcuma longa* and *Zingiber officinale* Rhizomes Ethanolic Extract

The rhizomes of *Curcuma longa* were washed under a running tap, cut into tiny pieces and dried at room temperature for 72 hours. An electronic weighing balance was used to measure 20 g, 40 g and 60 g of the powdered *C. longa* and each of the powdered *C. longa*, (20 g, 40 g and 60 g) was macerated into 100 mL of absolute ethanol for 72 hours after which it was then filtered using Whatman No.1 filter paper. The filtrate was properly labeled and stored in the refrigerator at 4℃ until when needed [17]. The same method was used for the maceration of *Zingiber officinale*.

2.4 Antibacterial Test of *Curcuma longa* and *Zingiber officinale* Rhizomes Ethanolic Extract

From 48 hours old culture of the test organisms, 0.5 mL of each test organism was inoculated in five different petri dishes using a micro pipette. About 20 mL of sterile media (Nutrient agar) was aseptically poured into each petri dish and the dishes were rocked for proper mixture. The media was allowed to solidify after which four wells were dug on each plate with the use of a 6 mm diameter sterilized cork borer. Three wells were for 20 g, 40 g and 60 g of powdered *C. longa* rhizomes/100 mL absolute ethanol and the fourth well was for ethanol which was used as the control. The wells were properly labeled and 0.5 mL of the *C. longa* rhizome ethanolic extract was dispensed into the first three holes and 0.5 mL of ethanol was dispensed into the fourth hole which served as the control. They were allowed to stay for an hour for proper diffusion of the extract into the media after which the plates were incubated at 37℃ for 24 hours. The effect of the *C. longa* ethanolic extract on the test organisms were indicated by clear zones of inhibition around the wells in the petri dishes. The diameters of the clear zones of inhibition were measured in millimeter using a transparent ruler. The same method was used for the antibacterial inhibitory test for *Z. officinale* rhizome ethanolic extract.

2.5 Antibiotic Sensitivity Test

The Kirby-Bauer method [18] was used to test the effects of different antibiotics on the test organisms (Bacterial isolates) and used to compare and contrast the antimicrobial activities of *Curcuma longa* and *Zingiber officinale.*

About 8 g of Diagnostic Sensitivity Test Agar (DSTA) was weighed using a clear electronic weighing balance and poured into a conical flask containing 200 mL of distilled water. The solution was stirred then sterilized in an autoclave at 121℃ for 15 minutes after which it was allowed to cool then poured into five different sterile plastic petri dishes and allowed to solidify. Each test organism was evenly spread on the media surface on separate petri dish using a sterile swab stick and allowed to stand for 1 minute after which an antibiotic sensitivity disc impregnated with various antibiotics namely; Ceftaxidime, Cefuroxime, Gentamycin, Ciprofloxacin, Oflaxacin, Ampicillin, Augmentin, Imipenem, Nitrofurantoin and Ceftriaxone was placed on top with the use of a sterile forcep. The plates were incubated at 37℃ for 24 hours. A clear zone around the disc indicates inhibition.

2.6 Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of *C. longa* rhizome ethanolic extract was carried out by

macerating powdered *Curcuma longa* in absolute ethanol. The powdered *C. longa* were measured differently into 1.3, and 1.6 grams and macerated into 8.7 and 8.4 mL of absolute ethanol respectively for 72 hours. The extract obtained was used to determine the minimum inhibitory concentration of *C. longa* rhizomes ethanolic extract against the test organisms following the earlier method mentioned above. The least concentration of *C. longa* that show inhibitory effects on the test organism was taken as the minimum inhibitory concentration. The same method was used for the determination of minimum inhibitory concentration of *Zingiber officinale* on the same test organisms [19].

2.7 Statistical Analysis of Data

Data for each group were collected and summarized in a tabular and graph forms for each treatment group. Data were represented as the mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was first used followed by Bonferroni t-test *post hoc* comparisons to determine the significant differences at 95% ($P < 0.05$) using Primer (version 3.01).

3. RESULTS AND DISCUSSION

The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal, and antiviral agents with significant activity against infective microorganisms [18,20]. Several reports have been published that described the antibacterial and antifungal properties of different herbs and spices [21]. However, there is little information about the exact mechanism of their antimicrobial action [22,23,24].

The result of this study showed that *Z. officinale* rhizome ethanolic extract has a higher inhibitory activity on the test organisms than *C. longa* extract. *C. longa* rhizome at 20 g/100 mL ethanol inhibited the growth of three isolates (*E. coli*, *S. aureus* and *S. pyogenes*) out of the total of five isolates used for this study while 40 g and 60 g inhibited the growth of all the five isolates (*E. coli*, *S. aureus*, *P. mirabilis*, *K. pneumoniae* and *S. pyogenes*) used for this study (Table 1). At 40 g and 60 g *C. longa*, there was a significant ($P < 0.05$) increase in the zone of inhibition of *E. coli*, *S. aureus*, *P. mirabilis* and *K. pneumoniae* and no significant change for *S. pyogenes* when compared with 20 g (Table 1).

Z. officinale rhizome at 20 g/100 mL ethanol also inhibited the growth of three isolates (*E. coli*, *S. aureus* and *S. pyogenes*) out of the total of five isolates used for this study while 40 g and 60 g inhibited the growth of all the five isolates (*E. coli*, *S. aureus*, *P. mirabilis*, *K. pneumoniae* and *S. pyogenes*) used for this study (Table 2). At 40 g and 60 g *Z. officinale*, there was a significant ($P < 0.05$) increase in the zone of inhibition of *P. mirabilis* and *K. pneumoniae* and when compared with 20 g (Table 2).

The result also showed 40 g and 60 g of both *C. longa* and *Z. officinale* to be more active on all the test organisms with significant increase in the zones of inhibition of *E. coli, S. aureus, P. mirabilis,* and *K. pneumoniae* with no significant change for *S. pyogenes* when compared with 20 g (Tables 1 and 2). This result is in conformity with the study carried out by [20] where they found the antibacterial activity of *Z. officinale* extract, lime and garlic against broad range of bacteria including *Bacillus* spp*, S. aureus, E. coli and Salmonella* spp.

The result of this study also showed that gram-positive bacteria used in this study (*S. aureus* and *S. pyogenes*) were more susceptible to the extracts of both *C. longa* and *Z. officinale* rhizomes as compared to the gram negative ones and this could be due to the presence of an outer membrane that serves as an effective barrier in gram-negative species [25,26].

More so, at 60 g *Z. officinale* rhizome/100 mL ethanol, there was a significant ($P < 0.05$) increase in the zone of inhibition of *S. pyogenes* when compared with 20 g (Table 2).

1.3 g of both *C. longa* and *Z. officinale* rhizome ethanolic extract was the least concentration that inhibited growth of some test organisms. *Z. officinale* was more active on the test organisms (*E. coli, P. mirabilis* and *K. pneumoniae*) at 1.3 g concentration of the ethanolic extract when compared to *C. longa* at the same concentration (Table 3).

Also, all the selected human pathogenic bacteria used in this study (*E. coli, P. mirabilis, K. pneumoniae, S. aureus* and *S. pyogenes*) were more sensitive to Ciprofloxacin followed by Ceptriaxome and Ofloxacin. Augmentin was slightly active on only *S. aureus* while Ampicillin was not active on any of the test organisms (Table 4).

This study however revealed sensitivity activity at 40 g and 60 g for both *C. longa* and *Z. officinale* indicating their antibacterial properties which could be used as substitutes for antibiotics.

Table 1. Zones of inhibition (mm) elicited by *Curcuma longa* rhizome ethanolic extract against the test organisms

Human pathogenic bacteria	20 g	40 g	60 g
Escherichia coli	6.67 ± 0.67	10.00 ± 0.00 [a]	9.33 ± 0.67 [a]
Staphylococcus aureus	8.00 ± 0.00	11.33 ± 0.67 [a]	11.33 ± 0.67 [a]
Proteus mirabilis	0.00	9.89 ± 0.00 [a]	10.00 ± 0.00 [a]
Klebsiella pneumoniae	0.00	8.00 ± 0.00 [a]	10.00 ± 0.00 [a]
Streptococcus pyogenes	13.33 ± 0.33	13.67 ± 0.88	12.33 ± 0.67

Values are Mean ± SEM; n = 3; [a] Significantly different from 20 g at P < 0.05

Table 2. Zones of inhibition (mm) elicited by *Zingiber officinale* rhizome ethanolic extract on the test organism

Human pathogenic bacteria	20 g	40 g	60 g
Escherichia coli	5.00 ± 1.00	8.67 ± 0.67	9.33 ± 2.91
Staphylococus aureaus	11.33 ± 0.67	13.67 ± 0.88	13.33 ± 1.67
Proteus mirabilis	0.00	9.33 ± 1.33 [a]	6.67 ± 0.00 [a]
Klebsiella pneumoniae	0.00	9.33 ± 1.33 [a]	4.00 ± 0.00 [a]
Streptococcus pyogenes	11.33 ± 0.67	22.67 ± 2.91 [a]	14.00 ± 0.00

Values are Mean ± SEM; n = 3; [a] Significantly different from 20 g at P < 0.05

Table 3. Minimum inhibitory concentration of *Curcuma longa* and *Zingiber officinale* rhizome ethanolic extract on test organisms

Pathogens	Minimum inhibitory concentration (1.3 g)		Minimum inhibitory concentration (1.6 g)	
	Curcuma longa	***Zingiber officinale***	***Curcuma longa***	***Zingiber officinale***
Escherichia coli	0.20	0.70	0.00	1.00
Staphylococcus aureaus	0.00	0.00	2.00	0.50
Proteus mirabilis	2.00	3.00	4.00	6.00
Klebsiella pneumoniae	0.00	2.50	0.00	6.00
Streptococcus pyogenes	0.00	0.00	0.00	0.00

Table 4. Zones of inhibition (mm) of antibiotic discs on tested organisms

Pathogens	Zone of inhibition (mm)									
	AUG	**AMP**	**CET**	**CEF**	**CEP**	**CPR**	**GEN**	**OFL**	**IMP**	**NIT**
Escherichia coli	0.0	0.0	22.0	17.0	18.0	28.0	0.0	18.0	10.0	25.0
Staphylococcus aureus	0.0	0.0	0.0	0.0	2.0	26.0	17.0	12.0	23.0	0.0
Proteus mirabilis	0.0	0.0	15.0	0.0	14.0	27.0	16.0	20.0	20.0	2.0
Klebsiella pneumonia	6.0	0.0	0.0	17.0	20.0	24.0	14.0	18.0	16.0	27.0
Streptococcus pyogenes	0.0	0.0	0.0	0.0	3.0	25.0	18.0	20.0	18.0	19.0

AUG-Augmentin; AMP-Ampicillin; CET-Ceftaxidine; CEF-Cefuroxime; CEP- Ceptriaxome; CPR- Ciprofloxacin; GEN-Gentamycin; OFL-Ofloxacin; IMP-Imipenem; NIT-Nitrofurantain; 0.00-No inhibition

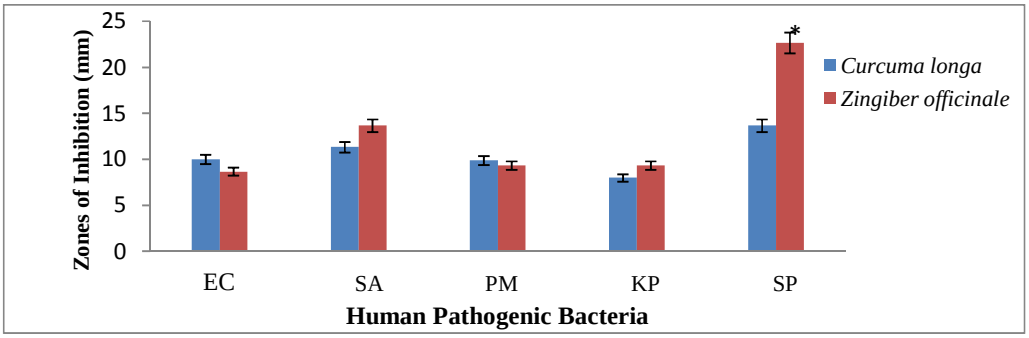

Fig. 1. Comparison of antibacterial activity of 40 g *Curcuma longa* and 40 g *Zingiber officinale* rhizome ethanolic extract on the tested human pathogenic bacteria

*Values are Mean ± SEM; n = 3; * Significantly different from 20 g at P < 0.05; EC-Escherichia coli; SA-Staphylococcus aureus; PM-Proteus mirabilis; KP-Klebsiella pneumoniae; SP-Streptococcus pyogenes*

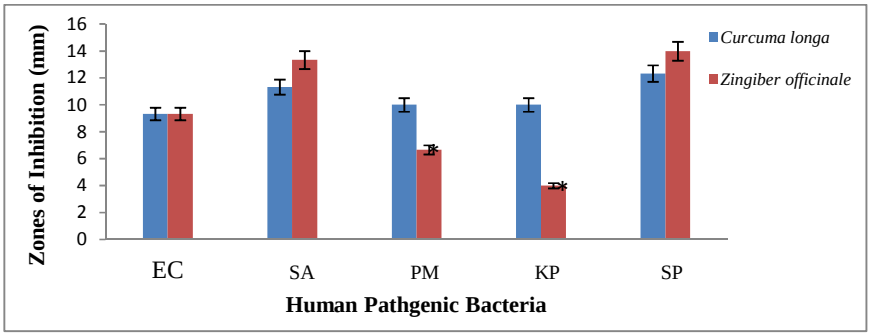

Fig. 2. Comparison of antibacterial activity of 60 g *Curcuma longa* and 60 g *Zingiber officinale* rhizome ethanolic extract against some human pathogenic bacteria

*Values are Mean ± SEM; n = 3; * Significantly different from 20 g at P < 0.05; EC-Escherichia coli; SA-Staphylococcus aureus; PM-Proteus mirabilis; KP-Klebsiella pneumoniae; SP-Streptococcus pyogenes*

The result of this study also showed that 40 g of *Z. officinale* rhizome ethanolic extract exerted a significant ($P < 0.05$) increase in the inhibition of the growth of *S. pyogenes* when compared with 40 g of *C. longa* (Fig. 1) while 60 g of *C. longa* rhizome ethanolic extract exerted a significant ($P < 0.05$) increase in the inhibition of the growth of *P. mirabilis* and *K. pneumoniae* when compared with 60 g of *Z. officinale* (Fig. 2).

4. CONCLUSION

In conclusion, *C. longa* and *Z. officinale* rhizomes were both sensitive to gram-positive and gram-negative bacteria used for this study and thus indicating their antibacterial properties which could be used as substitutes for antibiotics.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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