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DETERMINATION OF SYNERGISTIC ANTIBACTERIAL ACTIVITY OF GINGER (*ZINGIBER OFFICINALE)* AND GARLIC (*ALLIUM SATIVUM*) AQUEOUS EXTRACTS AGAINST CLINICAL RESISTANT *SALMONELLA TYPHI* ISOLATE

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ABSTRACT

In this study, fresh Ginger rhizomes (*Zingiber officinale*) and Garlic cloves (*Allium sativum*) were collected, washed, dried in the room and ground. The powders were added to distilled water for 72 hours. The extracts were filtered and four doubling concentrations (100, 50, 25 and 12.5 g/mL) were made each separate and combined. The extracts were applied against the *Salmonella typhi* isolate using the disc diffusion method. Ginger extract showed more activity with 24.67mm as the highest zone of inhibition at 100% g/mL. Garlic extract showed 11.33mm as zone diameter at 100% g/mL. The combined effect reduced the efficacy of the Ginger extract at all concentrations, the highest zone of inhibition recorded for the combined effect was 21.67mm at 100% g/mL. Tube dilution method was used to determine the Minimum Inhibitory

Introduction:

Microbial pathogenicity and other infectious diseases have been controlled by use of commercially available antimicrobial drugs for many years. Tremendous use of antibiotics has developed multiple drug resistance (MDR) in many bacterial pathogens. The increasing drug resistance is the main hindrance in successful treatment of infectious diseases and to the control of microbial pathogenicity (Fu *et al.,* 2007). Similarly, preservatives like sulfites, nitrates, nitrites and antibiotics are harmful for human health and have many side effects including headache, nausea, weakness, mental retardation, seizures,

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Concentration (MIC), various doubling concentrations (50, 25 12.5 and 6.25 g/mL) were used and the Ginger and Garlic extracts inhibited the *Salmonella typhi* except the least concentration (6.25 g/ml) which showed growth after the 24 hours incubation. The combined effect for the MIC also showed growth except at the highest concentration (50 g/mL). During the test for Minimum Bactericidal Concentration (MBC), all the plates showed growth after 24 hours incubation. This showed that the Garlic and Ginger have bacteriostatic effect against the test organism. Antibiotics sensitivity was also carried out with standard antibiotics discs (Cotrimoxazole (SXT) 30µg, Chloramphenicol (CH) 30µg, Sparfloxacin (SP) 10µg, Ciprofloxacin (CPX) 10µg, Amoxacillin (AM) 30µg, Augmentin (AU) 30µg, Gentamycin (CN) 10µg, Pepfloxacin (PEF) 30µg, Ofloxacin (OFX) 10µg and Streptomycin (S) 30µg) and compared with the antibacterial activity of the Ginger and Garlic extracts. The test organism may be multidrug resistant because, only Augmentin (30µg) and Sparfloxacin (10µg) were able to inhibit the bacterial growth with Sparfloxacin having the higher zone of inhibition of 25.00mm and Augmentin with 20.00mm.

Keywords: Synergistic, Antibacterial, Activity, Ginger (*Zingiber Officinale),* Garlic (*Allium Sativum*).

Cancer and anorexia (Rangan & Barceroux, 2009). Natural products are a major source of new natural drugs and their use as an alternative medicine for treatment of various diseases has been increased in the last few decades (Vuorelaa *et al.*, 2004). In comparison to the formulated drugs the herbs and spices have fewer side effects. They are also inexpensive, show better patient tolerance and are readily available for low socio-economic population (Adeshina *et al.*, 2011). In recent years, in view of their beneficial effects, use of spices or herbs is gradually increasing not only in developing countries but also in developed countries (Duman-Aydyn, 2008).

The antimicrobial activity of spices is due to specific phytochemicals or essential oils (Avato *et al.*, 2000). The main factors that determine the antimicrobial activity are the type and composition of the spice, amount

used, type of microorganism, composition of the food, pH value and temperature of the environment (Sagdic, 2003). Several reports had been published that describe the antibacterial and antifungal properties of different herbs and spices. However, still there is little information about the exact mechanism of their antimicrobial action (Gur *et al*., 2006).

Garlic (*Allium sativum*) in the family Liliaceae is a perennial bulb forming plant. It is world-wide known for dietary and medicinal purposes (Palaksha *et al*., 2010; Daka, 2011). Louis pasteur was the first to describe the antibacterial effect of onion and garlic juice (Penecilla & Magno, 2011).Garlic has been found to exhibit antibacterial activity against a wide range of Gram negative and Gram positive bacteria (Chechregni *et al*.,2011) including multidrug resistant strains and also possess antifungal activity and Anti parasitic activity (Farkhondeh *et al*.,2010).The most important chemical compounds of garlic which were thought to be responsible for antimicrobial activity are organosulphur compounds including allicin (Hovana *et al*., 2011). It showed better antibacterial activity than streptomycin and ampicillin (Ilic *et al*., 2012). Allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis. Although DNA and protein synthesis are partially inhibited suggesting that RNA is the primary target of Allicin action (Durairaj *et al*, 2009).The ability of garlic to inhibit the growth of both Gram positive and Gram negative bacteria shows that it has a broad spectrum of activity and can be used for formulation and development of newer broad spectrum antibacterial substances (Penecilla & Magno, 2011) and it provides scientific basis for its utilization in traditional and folk medicines (Meriga *et al*.,2012).Victor and Igeleke, (2012) found that garlic has very strong antimicrobial activity against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeroginosa* and *Salmonella typhi.*

Ginger is a member of the family Zingiberaceae; a small family with more than 45 genera, and 800 species; its scientific name is *Zingiber officinale* (*Z. officinale*) (Foster, 2011). It is an erect perennial plant growing from one to three feet in height; its stem is surrounded by the shealthing bases of the two ranked leaves. A clublike spike of yellowish, purple lipped flowers has greenish yellow bracts which rarely flowers in cultivation

(Tyler, 2002). Ginger is truly a world domestic remedy. It is also used in India and other places like the ancient Chinese where the fresh and dried roots were considered distinct medicinal products. Fresh ginger has been used for cold-induced diseases, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, less of appetite and rheumatism. In nineteenth century ginger serves as a popular remedy for cough and asthma when the juice of fresh ginger was mixed with a little juice of fresh garlic and honey (Foster, 2011). A paste of powdered dried ginger was applied to the temples to relieve headache and fresh ginger was mixed with a little honey, tapped off with a pinch of burnt peacock feathers to alley nausea.

Antibiotic resistance occurs naturally, but misuse of antibiotics in humans is accelerating the process (WHO, 2017). Also multidrug resistant pathogenic bacteria strains in the hospital and community are the main cause of mortality and morbidity (Osho & Bello, 2010). Hence, the use of natural drugs can be an alternative.

The aim of this work is to determine the synergistic antibacterial activity of Ginger and Garlic aqueous extracts against clinical pathogenic *Salmonella typhi* isolate.

METHODOLOGY

Study Area

The research was carried out in Bauchi at Abubakar Tafawa Balewa University, Bauchi and the practical was carried out in Microbiology laboratory of the university.

Preparation of Garlic and Ginger Extracts

The extracts were prepared according to the methods described by Abdulzahra and Mohammed, (2014). The Garlic bulbs and Ginger rhizomes (spices) were cleaned, peeled, sliced and dried at room temperature; the dried spices were ground to crucibles separately. Ten grams (10000mg) each of the Garlic and Ginger crucibles were weighed and put into two sterile conical flasks. Each 10000mg was dissolved in 50 milliliters of distilled water. The conical flasks were covered with cotton and left at 25°C

for 72 hours. The suspensions were filtered and the extracts were put into sterile containers and stored at 4°C until further use. The filtrates were considered as 100% concentration and three doubling concentrations were further made (50%, 25% and 12.5%) in the ratio 1:1.

Preparation of Garlic-Ginger Extract Solution

Equal volume of the Ginger and Garlic extracts were mixed to give 100% v/v and three doubling concentrations were made (50%, 25% and 12.5%).

Preparation of Mc-Farland's Standard Solution

McFarland's standard solution (1.5×10^8 CFU/mL) was prepared according to Andrews, (2006). Peptone water was prepared according to the manufacturer's instruction, the peptone water was inoculated with the bacterial isolate using a sterile wire loop and incubated aerobically at 37°C for 18-24 hours. One gram of barium chloride ($BaCl_2$) was dissolved in 99ml of distilled water and 1ml of sulfuric acid (H_2SO_4) was also dissolved in 99ml of distilled water which gave 100% for each. Then, 0.5ml was removed from the (H_2SO_4) solution and replaced with 0.5ml from the ($BaCl_2$) solution; this gave the McFarland's standard solution. Normal saline was prepared also by dissolving 1.7g of sodium chloride into 200ml distilled water; this was added to the inoculated peptone water until the turbidity was equal to the McFarland's standard solution.

Determination of Antibacterial Activity of the Extracts

Disc diffusion method was used to determine the antibacterial activity according to Kirby-Bauer, (1996). Discs of 6mm were punched from Whatmann's filter paper, the discs were put in Mc-Catney bottles and sterilized in hot-air oven, the discs were inserted into the different concentrations of the extracts with forceps and were allowed to soak, and the discs were later removed and dried to reduce the moisture content. Some discs were inserted into distilled water and maintained as negative control throughout the assay.

Mueller-Hinton agar medium was prepared according to the manufacturer's instruction; it was sterilized in an autoclave (121°C, 1 bar)

for 15 min and allowed to cool. 20ml each was poured into petri-dishes and were inoculated with 0.1ml of the McFarland's standard solution. The discs previously impregnated with the extracts were fixed into the cultured plates using sterile forceps and were incubated at 37°C for 18-24 hours. The zones of inhibition were examined and calculated in millimeters

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined by Broth dilution method according to methods described by Andrews, (2006). The extracts were diluted to four doubling concentrations ranging from 6.25-50% for Garlic, Ginger and a mixture of Garlic and Ginger. To each dilution of Ginger, Garlic extract and a mixture of both; 0.1ml of the standard clinical bacterial inoculum was inoculated. Negative control tubes with no bacterial inoculation were simultaneously maintained and were incubated aerobically at 37^0C for 18-24hrs. The MIC was recorded as the lowest concentration of the extracts that completely inhibits the growth of the organisms.

Determination of Minimum Bactericidal Concentration (MBC)

Dilutions showing no visible growth for the MIC were sub-cultured onto a fresh Mueller-Hinton (MH) agar plates and incubated at 37^0C for 18-24 hours. The lowest concentrations of the extracts with no visible growth on the MH plates were recorded as MBC (Patel *et al.*, 2011),

Determination of Antibiotics Susceptibility Pattern of the Isolate

The bacterial isolate was also examined for the sensitivity towards some commercially prepared antibiotics according to Kirby-Bauer, (1996). Commercially prepared discs impregnated with antibiotics which include; Cotrimoxazole (SXT) 30µg, Chloramphenicol (CH) 30µg, Sparfloxacin (SP) 10µg, Ciprofloxacin (CPX) 10µg, Amoxacillin (AM) 30µg, Augmentin (AU) 30µg, Gentamycin (CN) 10µg, Pepfloxacin (PEF) 30µg, Ofloxacin (OFX) 10µg and Streptomycin (S) 30µg were fixed with sterile forceps over Mueller-Hinton agar plate previously inoculated with the test organism.

The plates were placed in the incubator at 37^{0}C for 18-24 hours and the zones of inhibition were measured and recorded in millimeters.

RESULTS

The results obtained indicate that Garlic and Ginger may have some potential against pathogenic microorganisms like *S. typhi*. Figure 1 shows that Ginger is more effective against the isolate than Garlic.

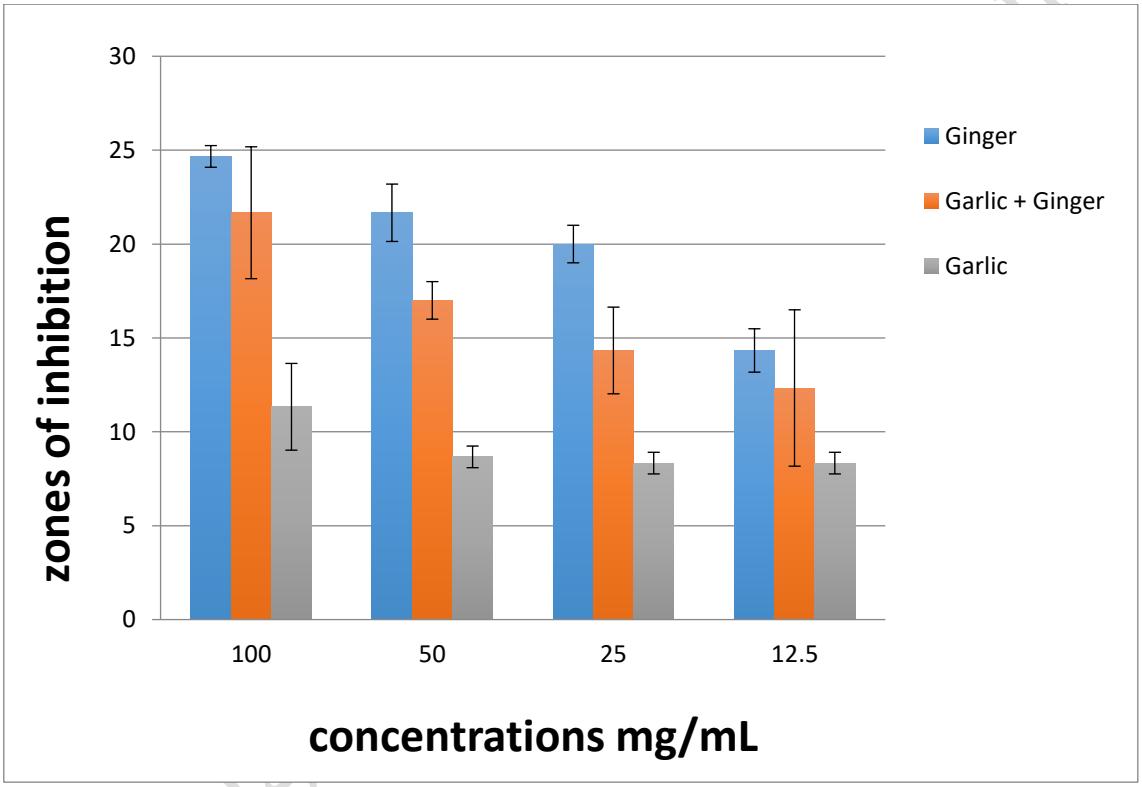

Figure 1: Antibacterial Activity of the Extracts against the test organism (Salmonella typhi).

Table 1: Minimum Inhibitory Concentration (MIC) of the Extracts against the test organism (*Salmonella typhi*)

Concentrations (mg/mL)	Garlic Aqueous Extract	Ginger Aqueous Extract	Ginger + Garlic Aqueous Extract
50	-	-	-
25	-	-	+
12.5	-	-	+

6.25	+	+	+

Key; No Inhibition (+), Inhibition (-)

Table 2: Minimum Bactericidal Concentration (MBC) from various concentrations of the MIC

Key; Growth (+)

Concentrations (mg/mL)	Garlic Aqueous Extract	Ginger Aqueous Extract	Ginger + Garlic Aqueous Extract
50	+	+	+
25	+	+	+
12.5	+	+	+

Table 3: Antibiotics sensitivity test of the isolate (*Salmonella typhi*) with zones of inhibition in millimeter (mm)

Serial number	Antibiotics	Zones of inhibition (mm)
1	Amoxacillin (30µg)	6.00
2	Augmentin (30µg)	20.00
3	Chloramphenicol (30µg)	6.00
4	Ciprofloxacin (10µg)	6.00
5	Gentamycin (10µg)	6.00
6	Pepfloxacin (30µg)	6.00
7	Cotrimoxazole (30µg)	6.00
8	Sparfloxacin (10µg)	25.00
9	Streptomycin (30µg)	6.00
10	Ofloxacin (10µg)	6.00

Data analysis

The results obtained for the antibacterial activity of the Garlic and Ginger extracts were interpreted with Microsoft Office Excel 2010.

DISCUSSION

The results obtained above indicate that Garlic and Ginger may have some potential against pathogenic microorganisms like *S. typhi*. Figure 1 shows that Ginger is more effective against the isolate than Garlic. This may be as

the result of the phytochemical constituents of the Ginger rhizomes, the major phytochemicals include; carbohydrates (50-70%) lipids (3-8%), terpenes and phenolic compounds.

The terpenes components of Ginger include; Zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene and α-curcumene, while phenolic compounds include gingerol, paradols and shogaol. The gingerol (23-25%) and shogaol (18-25%) are found in higher quantity than others which is the cause of the higher antibacterial activity, other phytochemicals that are also found in the Ginger rhizomes include amino acids, raw fibres, ash, protein, phytosterols, vitamin and mineral are also present (Prasad & Tyagi, 2015).

Garlic was reported to contain allicin (allyl 2-propene thiosulfinate); a notable flavonoid (Ross *et al.,* 2000). Garlic also has sulphur-containing compounds such as alliin, ajoene, diallylsulphide, dithin, S-allylcysteine, enzymes and other non sulphur-containing compounds like vitamin B, proteins, minerals, saponins and flavonoids (Johnson *et al.*, 2008).

The addition of Garlic aqueous extract reduced the effect of Ginger aqueous extract at each concentration and the combined effect is less than the effect of Ginger alone against the isolate (*S. typhi*). Ocampo, (2014) reported that antibacterial combination can be synergistic or antagonistic.

Abdulzahra & Mohammed, (2014) highlighted that Ginger and Garlic have synergistic effect against bacteria such as; *Staphylococcus aureus, Klebsiella pneumonia, Streptococcus pyogens, Pseudomonas aeruginosa & Proteus mirabilis* with a highest zone of inhibition of 12mm for the aqueous solution.

The antibacterial activity of Garlic and Ginger was also reported against some species of bacteria which include; *Escherichia coli, Bacillus cereus, Staphylococcus aureus and Salmonella typhi*, with Garlic showing more efficacy than Ginger against the test organisms. Ginger did not show any activity against *Salmonella typhi* for both agar well diffusion and disc diffusion methods. The highest zone of inhibition was from Garlic extract in agar well diffusion with 38mm against *Staphylococcus aureus,* and 26mm was recorded for Garlic extract in disc diffusion method against *Bacillus cereus* (Chand, 2013).

Ekwenye and Elegalan, (2005) also reported the antibacterial efficacy of Garlic and Ginger (aqueous and ethanolic extracts) against *Escherichia coli* and *Salmonella typhi* with 8mm diameter of inhibition recorded from Ginger aqueous extract against *S. typhi* only, with no diameter of inhibition recorded from Garlic aqueous extract.

The mean zones of inhibition increase with increase in concentration in each case of the Ginger extract, Garlic extract and the mixture of both. The concentration with the highest zone of inhibition is 100% of the Ginger extract with zone diameter of 24.67 mm; while the concentration with the lowest zone of inhibition is 12.50% of the Garlic extract with zone diameter of 8.33 mm. Discs of 6mm were punched, soaked in distilled water and maintained as negative controls throughout the antibacterial test of the extracts.

Both Ginger and Garlic extracts showed no visible growth when tested for MIC except for the least concentration (6.25%) as seen from Table 1 and for the combined effect, only the highest concentration (50%) did not show visible growth. Negative control tubes with no bacterial inoculation were also maintained for the MIC test. The tubes that showed growth for the MIC were sub cultured into newly prepared Mueller-Hinton agar plates and were tested for the Minimum Bactericidal Concentration (MBC). As shown in Table 2, all the plates showed visible colonies after an incubation of 18-24hours, this also showed that the antibacterial effect of the extracts may be static (not cidal).

When tested for sensitivity of the isolate towards some synthetic antibiotics (impregnated antibiotics discs) that are known to be active against gram negative bacteria, only Augmentin (30µg) and Sparfloxacin (10µg) were able to inhibit the bacterial growth with Sparfloxacin having the higher zone of inhibition of 25.00mm as shown in Table 4.

The isolate prove to be resistant against the remaining (8) antibiotics discs which indicates it may be multi-drug resistant (MDR) and most synthetic antibiotics are no longer effective against pathogenic *Salmonella typhi*.

CONCLUSION

The project highlighted that Garlic and Ginger (plant materials) may have some potential against pathogenic microorganisms such as *S. typhi*. Ginger

and Garlic may serve as alternative to the synthetic antibiotics in which most of them were seen to be resistant. The combination of Garlic and Ginger showed clear zones of inhibition against the test organism, although Ginger extract showed higher activity than Garlic extract.

Furthermore, the research was able to demonstrate that natural products may be alternative to the formulated drugs and may even be better because they are less toxic, cheaper and reduce the chances of developing drug resistance by pathogenic microorganisms as the result of misuse of synthetic antibiotics.

RECOMMENDATIONS

Based on the results obtained the following recommendations are made;

1. The use of natural products may serve as alternative to resistance seen to antibiotics.
2. Further research is suggested to investigate and extract the active ingredients that are responsible for the antimicrobial property of Garlic and Ginger so as to formulate antibiotics that are from natural products.
3. Mechanism of action of the Ginger and Garlic could also be investigated.

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