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Synergistic Effect of Allium sativum (garlic) and Zingiber officinale (ginger) against Escherichia coli and staphylococcus aureus

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Abstract --The soxhlet ethanolic extracts of Garlic (Allium sativum) and Ginger (Zingiber officinale) were subjected to phytochemical screening. The result revealed the presence of Alkaloids, Saponins, Cardiac glycosides, Steroids and Flavonoids in both plants while Tannins was absent in Allium sativum but present in Zingiber officinale. The antibacterial activity of the plant extracts was assayed by the agar well diffusion method. The test isolates were Staphylococcus aureus and Escherichia coli both of which are clinical isolates. Both extracts had strong antibacterial activity against the isolates with Escherichia coli having the highest zone of inhibition with Garlic (24.00mm) and Staphylococcus aureus with Ginger (28.00mm).the minimum inhibitory concentration of both extracts was 10-2 while the minimum bactericidal concentration of both extracts was 10-1. The synergistic effect of both extracts gave a stronger activity with Staphylococcus aureus being more susceptible with a zone of inhibition of 39.00mm and Escherichia coli having 34.60mm. This confirms the use of the plant extracts in the treatment of ailments caused by these microorganisms.

Keywords: *S aureus, E.coli, Allium sativm, Zingiber officinale*, Phytochemicals, antibacterial.

INTRODUCTION

Medicinal plant is a therapeutic resource used by the traditional population of the African continent specifically for health care and which may also serve as precursors for the synthesis of useful drugs (Sofowora, 1993). The World Health Organisation (WHO) defined a medicinal plant as any herbal preparation produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which are produced for immediate consumption or a basis for herbal products (WHO2001). The term herbal drug determines the part or parts of plant used for preparing medicines (example leaves, flowers, seeds, roots, barks, stems e.t.c). Practitioners of traditional medicine believe that the constituents of plants are unique as they contain both active and non active components that play a role in enhancing the well-being of their patients. A rekindle interest in the pharmaceutical importance of plants have led to the discovery and adoption of plant extracts which were commonly used in traditional medicine, as alternative source of remedy. Numerous plants and herbs are used all over Nigeria by traditional medicine practitioners. Studies on the use of plants extract for the control of diseases have shown the importance of natural chemicals (phytochemicals) as possible sources of non-phytotoxic and easily biodegradable alternative fungicides and antibiotics (Akueshi et al., 2002).

Ginger (Zingiber officinale) is an underground stem, or a rhizome of the family Zingibereceae, a herbaceous perennial plant which consists of erect leafy shoots (Chandarana et al., 2005). It has strong antibacterial and to some extent, antifungal properties. It inhibits the growth of Escherichia coli, Proteus spp, Staphylococci, Salmonella spp (James et al., 1999). Ginger also inhibits Aspergillus a fungus known for production of aflatoxin, a carcinogen (Kapour 1997). Ginger therefore, helps prevent or treat nausea and vomiting from motion sickness, and morning sickness (pregnancy sickness). It is also used as digestive aid for mild stomach upset, reduce pain of osteoarthritis, and may even be used in heart disease or cancer treatment.

Garlic (Allium sativum) a specie in the genus Allium. Allium, its close relative that include the onion, shallot, leek, chives (Block, 2010). It has long been a staple food in the Mediterranean region, as well as a frequent seasoning in Asia, Africa and Europe. Garlic has been used throughout its history for both culinary and medicinal purposes (Gualtero et al., 1990). Garlic exhibit a broad antibiotic activity against both gram positive and gram negative bacteria. Such as Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, proteus spp, Bacillus cereus, Klebsiella, Bacillus substilis. Garlic cloves are used as remedy for infection (especially chest problems) and digestive disorders (Gualtero et al., 1990).

Phytochemicals are non-nutritive plant chemicals that have protective or disease precaution properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life, it is well known that plants produce these chemicals to protect themselves, but recent research demonstrate that they can also protect humans against diseases. There are more than 10,000 known phytochemicals, some of the well-known ones include: tannins, saponins, flavonoids, cardiac glycosides, steroids, alkaloids, carotenoids e.t.c. (Hill 1992)

The aim of this paper is to extract the active components of ginger and garlic, determine the phytochemicals present, the antibacterial activity and synergistic effect of the extracts on clinical isolates of Escherichia coli and Staphylococcus aureus.

MATERIALS AND METHODS

Sample collection

The fresh plants (garlic and ginger) were air dried at room temperature for 3 weeks and then pulverized separately using mortar and pestle to fine particles. 450g of both garlic and ginger powder was weighed and stored in an air tight container and kept at room temperature until required.

Clinical isolates of Escherichia coli and Staphylococcus aureus were procured from microbiology laboratory of Yusuf Dantsoho Memorial Hospital Tudun-wada Kaduna.

Extraction of plant materials

50g of both garlic and ginger were separately wrapped in a sterile cotton wool cloth. It was then placed into a thimble of the soxhlet apparatus using a pair of forceps. The distillation flask was filled with 250cm3 of 95% ethanol coupled to the extraction unit and placed on a water bath. The condenser unit was then mounted on the extractor unit connected to a running tap. The preparation was allowed to reflux for 4hrs after which the ethanol in the thimble turned faint green, indicating that the extraction has been completed. Thereafter, the thimble was carefully removed when the distillation flask was almost free of ethanol. The extract in the conical flask was placed in a water bath to concentrate it and to obtain the crude extract. The crude extract was allowed to cool and was weighed and recorded.

Phytochemical screening

The phytochemical analysis of the garlic and ginger was carried out following the methods described by Trease and Evans (1989).

Preparation of Mcfarland standard

Barium sulphate (190w/v) standard suspension was used as turbidity standard. It was prepared by adding 1ml of concentrate H2SO4 in 99ml of water. 1g of barium chloride was dissolved in 100ml H2SO4 acid solution to yield 1.0%w/v barium sulphate solution. The turbid solution that was formed was then transferred into a test tube as the standard for comparism (Chessbrough, 2004). This match with the 0.5 Mcfarlands standard turbidity to prepare using the test bacteria culture. Exactly 0.5 Mcfarland gives equivalent approximate density of bacteria 1.5x10-8 cfu (Chessbrough, 2004).

Standardization of inoculum

The previously prepared overnight nutrient broth culture of each bacteria were used as innocula by diluting with sterile saline solution. The sterile normal saline was prepared by weighing 0.5g of NaCL and dissolved into 100cm3 of sterile distilled water. 10ml of the solution was transferd to cool, and 0.1ml of each of overnight broth culture of Staphylococcus aureus and Escherichia coli was dispensed into the separate test tubes (20) containing the sterile normal saline. The suspension was adjusted to match the 0.5 Mcfarlands standard which has a similar appearance of an overnight broth culture. This served as the standard innocula which was used for the antibacterial activity testing and for the determination of minimum inhibitory concentration and minimum bactericidal concentration of the extracts.

Antibacterial activity

The antibacterial activity was conducted using the method described be Al-Mahmood (2009). The stock was prepared by dissolving 2g of each crude extract in 2ml of distilled water to make a stock concentration of 1000mg/ml. From the stock, serial dilutions was made from 10-1 to 10-4. The sterilized Muller Hinton agar was poured into sterile Petri dishes and allowed to solidify. A sterile swab stick was dipped into the standardized innocula and spread on the solidified nutrient agar aseptically and labelled. The inoculated plates were allowed to stay for 30 minutes to enable the organisms stick properly to the surface of the agar. Five wells were bored aseptically with the use of a sterile cork borer of 6mm diameter. The wells were then filled with 0.1ml of the serially diluted solution of each extracts. Ciprofloxacin was used as a positive control. The plates were incubated at 370c for 24hours, after which zones of inhibition were observed, measured and recorded in milimeter.
Synergistic test
Equal volumes of the extracts were combined by measuring 2ml each from the different dilutions into four test tubes each and mixed thoroughly. This was used for the synergistic test following the same procedure for the antibacterial activity.
Determination of minimum inhibitory concentration (MIC) of the extracts
The determination of the minimum inhibitory concentration (MIC) of the plant extracts was carried out. 0.1ml of the standard innocula of the test organisms were inoculated into a sterile nutrient broth containing the different concentrations of the plant extracts. The test tubes were incubated at 37oC for 24hours and observed for turbidity. The lowest concentration at which no detectable bacterial growth was recorded as the MIC.

Determination of the minimum bactericidal concentration (MBC) of the extracts

The minimum bactericidal concentration was determined from the broth dilution test resulting from the minimum inhibitory concentration tubes by inoculating the content of each test tubes on a nutrient agar plates. The plates were then incubated at 37oc for 24hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration (MBC).

SSRESULTS
The phytochemical screening of the ethanolic crude extract of Allium sativum (garlic) and Zingiber officinale (ginger) is shown in Table 1. The result revealed the presence of alkaloids, saponins, cardiac glycosides, steroids and flavonoids in both plants while tannins was absent in Allium sativum but present in Zingiber officinale.

Table 1: Phytochemical screening of ethanolic extract of Allium sativum and Zingiber officinale.

Phytocomponents	Allium sativum	Zingiber officinale
Alkaloids	+	+
Saponins	+	+
Cardiac glycosides	+	+
Steroids	+	+
Tannins	_	+
Flavonoids	+	+

Key: + = positive - = negative

The antibacterial activity of the ethanolic extract of Allium sativum and Zingiber officinale against Staphylococcus aureus and Escherichia coli is shown in Table 2. The result shows zone of inhibition of the extracts of the plants at different concentrations against the clinical isolates. Staphylococcus aureus has a range of 3.59-15.80 and 13.00-28.00 for Allium sativum and Zingiber officinale respectively. Escherichia coli has a range of 10.24-24.00 and 14.50-23.56 for Allium sativum and Zingiber officinale respectively.

TABLE 2: Antibacterial activity of ethanolic extract of Allium sativum and Zingiber officinale

Zone of inhibition (mm)									
Organism	Allium sativum				Zingiber officinale				cipro
	Concentration								
S. aureus	10-1	10-2	10-3	10-4	10-1	10-2	10-3	10-4	500mg
1	15.50	10.00	08.24	06.00	28.00	23.09	20.79	15.00	29.00
2	13.20	09.00	07.24	05.56	25.13	21.18	17.60	13.23	28.50
3	11.50	08.80	05.28	03.59	26.74	20.46	18.71	13.12	28.00
4	15.10	09.70	08.13	06.66	28.00	22.84	18.80	14.11	28.00
5	15.00	10.13	07.99	06.94	27.92	22.91	17.45	15.26	29.00
6	12.40	11.83	08.26	05.24	24.14	22.60	17.33	15.14	28.75
7	15.00	13.79	10.74	08.34	26.28	23.12	20.25	14.32	29.10
8	10.50	09.14	08.00	07.56	27.65	20.24	19.00	14.40	28.95
9	11.22	10.24	08.14	05 21	24.94	20.18	17.00	13.50	28.86
10	15.80	12.88	10.16	08.17	24.45	21.42	19.50	13.00	29.00
E.coli									
1	20.00	18.24	15.25	13.56	23.00	20.50	18.50	16.00	30.20
2	21.50	17.00	13.00	10.79	22.14	20.40	18.46	15.60	30.00
3	23.00	20.11	18.50	15.67	23.56	19.40	18.34	15.00	30.15
4	24.00	21.24	19.11	17.13	22.60	19.25	17.60	16.25	30.10
5	20.00	17.50	15.00	12.00	21.62	20.56	16.74	16.50	30.05
6	19.23	16.14	13.24	10.45	22.15	20.78	17.00	14.50	30.20
7	19.23	17.00	15.50	12.00	22.14	19.85	18.14	17.00	30.16
8	18.10	15.24	13.50	11.50	21.13	19.60	18.15	17.00	30.18
9	17.24	16.00	12.32	10.24	22.15	20.11	17.12	15.00	30.08
10	16.00	17.00	14.56	11.00	22.20	20.15	17.13	16.15	30.10

The result of the synergistic effect of the plants extracts is shown in table 3. The combination of the two plants extracts had a

higher zone of inhibition when used together compared to using the plants extract individually.

Table 3: Synergistic effect of Allium sativum and Zingiber officinale on Staphylococcus aureus and E. coli.

Organism	Zone of inhibitions (mm) Synergism of Allium sativum and Zingiber officinale Concentration			
S. aureus	10-1	10-2	10-3	10-4
1	37.34	34.56	32.00	29.40
2	35.56	33.25	31.25	29.15
3	38.28	33.33	32.14	28.26
4	36.43	35.40	30.30	27.30
5	35.40	33.24	30.15	28.33
6	37.60	34.50	30.00	28.50
7	37.71	34.16	31.00	27.12
8	38.13	34.28	31.17	29.64
9	37.12	33.40	32.20	28.18
10	39.00	35.21	32.50	28.39
E. coli				
1	30.00	29.79	26.50	24.18
2	33.23	29.80	26.60	24.34
3	34.50	29.76	26.54	23.50
4	34.60	28.46	25.12	22.00
5	33.79	27.48	25.15	23.00
6	34.25	28.35	26.29	33.57
7	33.26	28.50	26.48	24.60
8	33.35	27.50	25.32	20.18
9	34.46	27.15	25.85	20.18
10	34.55	27.68	25.20	20.29

The minimum inhibitory concentration (MIC) of the ethanolic extracts of Allium sativum and Zingiber officinale is shown in table 4. The MIC was observed at 10-2 for all the organisms which indicated that the extracts were able to inhibit the growth of the organisms at that concentration therefore no turbidity was observed.

Table 4: Minimum Inhibitory Concentration (MIC) of ethanolic extract of Allium sativum and Zingiber officinale.

Organisms	Allium sativum				Zingiber officinale			
	Concentrations							
S. aureus	10-1	10-2	10-3	10-4	10-1	10-2	10-3	10-4
1	-	-	+	+	-	-	+	+
2	-	-	+	+	-	-	+	+
3	-	-	+	+	-	-	+	+
4	-	-	+	+	-	-	+	+
5	-	-	+	+	-	-	+	+
6	-	-	+	+	-	-	+	+
7	-	-	+	+	-	-	+	+
8	-	-	+	+	-	-	+	+
9	-	-	+	+	-	-	+	+
10	-	-	+	+	-	-	+	+
E. coli								
1	-	-	+	+	-	-	+	+
2	-	-	+	+	-	-	+	+
3	-	-	+	+	-	-	+	+
4	-	-	+	+	-	-	+	+
5	-	-	+	+	-	-	+	+

6	-	-	+	+	-	-	+	+
7	-	-	+	+	-	-	+	+
8	-	-	+	+	-	-	+	+
9	-	-	+	+	-	-	+	+
10	-	-	+	+	-	-	+	+

The minimum bactericidal concentration (MBC) of the ethanolic extract of Allium sativum and Zingiber officinale is shown in table 5: The minimum bactericidal concentration of both plants was observed at the 10-1 for all the organisms indicating no growth on nutrient agar plant.

Table 5: Minimum Bactericidal Concentration (MBC) of ethanolic extract of Allium sativum and Zingerber officinale.

Organism	Allium sativum		Zingiber officinale	
	Concentration			
S. aureus	10-1	10-2	10-1	10-2
1	-	+	-	+
2	-	+	-	+
3	-	+	-	+
4	-	+	-	+
5	-	+	-	+
6	-	+	-	+
7	-	+	-	+
8	-	+	-	+
9	-	+	-	+
10	-	+	-	+
E. coli				
1	-	+	-	+
2	-	+	-	+
3	-	+	-	+
4	-	+	-	+
5	-	+	-	+
6	-	+	-	+
7	-	+	-	+
8	-	+	-	+
9	-	+	-	+
10	-	+	-	+

Key: + = Growth
- = No growth

DISCUSSION

The result of the phytochemical screening revealed the presence of alkaloids, saponins, cardiac glycosides, steroids, and flavonoids in both garlic and ginger, but tannins was absent in garlic but present in ginger which agrees with the findings of Abirosh et al., 2006. The presence of phytochemicals is responsible for the medicinal properties of the extracts. Studies have shown that saponins, tannins, flavonoids, and phenolic compounds possess antimicrobial activities (Mboto et al., 2009).

The antibacterial activity of the extracts against the test isolates shows that the extracts of garlic and ginger have inhibitory effect against the test isolates. Garlic produced zones of inhibition range of 03.59-15.80mm and 10.24-24.00mm for Staphylococcus aureus and Escherichia coli respectively which is similar to the findings of Amagase, 2006 who had 02.29-15.20mm and 10.10-24.80mm. Ginger extract had zone of inhibition of 13.00-28.00mm and 14.50-23.56mm respectively.

The level of performance between garlic and ginger and their combination was determined by ANOVA method which shows that garlic has mean zone of inhibition of 19.83mm and 13.52mm. Ginger has 22.77mm and 26.32mm and the combined extract 33.60mm and 37.25mm for both Staphylococcus aureus and Escherichia coli respectively. This shows that the mean difference between garlic, ginger and their combination is statistically significant with P value of 0.000. The combination of garlic and ginger has the highest zone of inhibition this agrees with the findings of Onyeagba et al., 2004 who found the synergistic effect of ethanol extract of ginger and garlic against Bacillus spp and Staphylococcus aureus. There is statistical significant difference between garlic and ginger as regard to their zones of inhibition with P value of 0.000 with ginger being the most efficacious.

The result of the MIC revealed that both extracts were able to inhibit the growth of both organisms at 10-2. While the MBC result showed that both extract were able to kill the organisms at 10-1 concentration for both organisms. This concentration is important because it is usually used to evaluate the efficacy of agents such as antiseptics, disinfectants and chemotherapeutics.

CONCLUSION

The study has demonstrated the effectiveness of garlic and ginger on Staphylococcus aureus and Escherichia coli, but their combination was more effective against the test isolates.

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