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Antimicrobial resistance profile and antibacterial activity of ginger and garlic extract on diary isolated *E. coli* And *Salmonella typhii*

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Abstract

This study investigated the *in vitro* comparative assessment of antibacterial activity of ginger (*Zingiber officinale*) and garlic (*Allium sativum)*. Four different extracts were prepared (ethanol, n-hexane, ethanol + waters, aqueous extracts) of ginger and garlic to determine the antibacterial action against rising spates of resistance to *Escherichia coli* and *Salmonella spp.* which were isolates from raw milk of cattle using disc diffusion method. The bacterial strains used was identified and characterized by standard bacteriological method (colonial morphology, biochemistry), PCR and Sanger Sequencing Techniques (molecular). Both garlic and ginger were identified at FRIN in Ibadan and then concentrated at Department of Pharmaceutical Chemistry, University of Ibadan. The extracts of both ginger and garlic showed remarkable resistance against both organisms with no inhibitory zone while the synthetic antibiotic used as control (Chloramphenicol) was sensitive against the two test microorganisms. The result obtained was subjected to statistical ANOVA at P≥ 0.05 level of significance. Although both plants have been reported to have anti-bacterial properties but this study contrasted this assertions making its recommendation as plants of choice in ameliorating bacterial infection as a cause for re-examination.

Keywords: Ginger (*Zingiber officinale*); Garlic (*Allium sativum*); Anti-bacterial; Assessment; Disc diffusion

1. Introduction

Ginger (*Zingiber officinale***),** Roscoe belonging to the Family *Zingiberaceae*, is a perennial herb with thick tuberous rhizomes. The erect leafy aerial stem grows up to approximately 1 meter in height and has purple. Its roots are used as spice in cooking throughout the world. The ginger plant has a long history of cultivation known to originate in China and then spread to India, South East Asia, West Africa and the Caribbean [1]. Mature ginger roots are fibrous and nearly dry. They can be cooked as an ingredient in many dishes. They can be stewed in boiling water to make ginger tea, to which honey is often added as a sweetener; sliced orange or lemon fruit may also be added. Ginger is also made into candy and used as flavoring for cookies, crackers and cakes as well as favour in ginger ale-a sweet, carbonated, non-alcoholic beverage. Ginger bread, ginger snaps, ginger cake and ginger biscuits [1]. Ginger has been reported to be effective for the treatment of inflammation, rheumatism, cold, heat cramps, and diabetes [2]. Ginger effect on the oral bacteria, crude extract of the Ginger can inhibit the growth of oral bacteria *in vitro* [3]. Ginger extract showed antimicrobial activity [4]. In recent years, in view of their beneficial effects, use of spices or herbs is gradually increasing

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not only in developing countries but also in developed countries [5]. The antimicrobial activity of spices is due to specific phytochemicals or essential oils [6].

Garlic is the common name of the genus *Allium sativum* the other closely related species including the shallot, the onion and others. *Allium sativum* can be considered as a national product of many centralized Asian countries. There are various kinds or subdivisions of *Allium sativum*, more obviously soft neck and hard neck garlic. Hard neck garlic has been usually developed in nearly cool weather while soft neck garlic is usually well developed in close vicinity to the equability line. In new researches, it is reported that garlic extract has been proved to be efficient toward *Streptococcus mutans;* garlic extract mouth wash may be utilized as a modern line in inhibiting dental caries formation. Garlic *(Allium sativum)* has been used as spice and primitive medicine. It has possessed antibacterial, antifungal, anti-parasitic, antiviral, antioxidant, anti-cholesteremic and vasodilator characteristics [7]. Besides that, garlic may have the ability to prevent and manage viral, fungal and even helminthes infections. Newly obtained garlic has been found to impart a significant role in managing food poisoning through killing the causative agents such as *Escherichia coli*. Recently, it has approved that there is a possibility of using garlic in preserving meats from bacterial spoilage and this related to the antibacterial activity of garlic. Upon treatment of meat with garlic, bacterial numbers were significantly diminished in comparison with the non-treated meat when both meats were kept in refrigerator at 4°C.Garlic is highly documented in exhibiting powerful antimicrobial activities. A similar study [8] discovered that both ginger and garlic produced marked inhibitory effect on *S. typhii* and *E. coli* as representative of enteric microorganisms although this was dependent on the solvents used for the extractions.

2. Material and methods

2.1. Collection and Identification of Plant Material

Fresh ginger (*Zingiber officinale*) and garlic (*Allium sativum)* were purchased from Sango market in Saki, Oyo State, Nigeria. The fresh leaf samples of the plants were collected and identified at Forestry Research Institute of Nigeria (FRIN), Ibadan and later confirmed at the Herbarium section with the specimen voucher number of 11502.

2.1.1. Drying and Grinding of Plant Materials

The fresh ginger rhizomes and garlic were deskinned, washed for 5-6 times with sterile distilled water and air dried for 3 weeks. After drying, the ginger and garlic slices were pulverized into fine powder using electric blender and the resulting powder was packed into a clean plastic container with screw cap for subsequent analysis.

2.1.2. Preparation of Test Organisms

Different bacterial strains including *Escherichia coli*, *Salmonella spp.* were obtained were isolated from water, milk and identified to the species level by using different available procedures including Gram's stain, Biochemical test(Catalase test, Oxidase test).All isolates were checked for purity and confirmed by gram staining and sub culturing them on selective media which include Eosin Methylene Blue(E.M.B) and MacConkey agar ,thus, observing the colony characteristics and morphology of the cells and incubated at 37°C overnight. Further identification was performed by using PCR and Sanger Sequencing Techniques.

2.1.3. Preparation of the Extracts

Four different solvents (aqueous, ethanol, ethanol + water and n-hexane) were used to extracts ginger and garlic separately.100g powder of ginger and garlic were weighed into a sterile bottle and was soaked in 500ml of ethanol and n-hexane.100g of each powdered samples were also weighed into a sterile bottle and soaked with 250ml of ethanol plus 250ml of distilled water. The mixture were stirred carefully with sterile stirrer to get uniform sample and the bottles was later covered with aluminum foil paper. The bottles were kept at room temperature for 72 hours (3 days). The mixture extracted were sieved through sterile muslin cloth, cotton wool and later filtered with Whatman filter paper. The powdered samples of 150 g was soaked with 750ml of distilled water in the night of extraction. It was stirred gently and boiled for 15 mins. It was allowed to cool and was extracted in the same way as above. The extracts were then transported to the Department of Pharmaceutical Chemistry Laboratory in University of Ibadan, Oyo State for concentration.

2.2. Determination of the Antibacterial Activity of Plant Extract

2.2.1. Preparation of Minimum Inhibitory Concentration of the Extract

Filter paper was sterilized and perforated into disc of 6 mm sizes. The concentration was diluted into 100 ml of each solvent (ethanol, n-hexane, distill water, ethanol + water) in a measuring cylinder and was stirred gently with sterile stirrer until it was dissolved into liquid form. 75 ml of the 100 ml of the solvents was poured into another measuring cylinder and 25ml was poured to make it 100 ml. The paper disc was soaked with different concentration (5ml/g, 10ml/g, 15ml/g, 20ml/g and 25ml/g) volume of the extract using micropipette and control was also soaked with the same volume and was placed on the plate. Each test organisms were streak on 14 different Mueller Hinton agar plates under aseptic condition and were labeled accordingly. The plates were incubated at 37°C for 24 hours. The zone of inhibition was measured in millimeter (mm) using Vernier Caliper or meter rule and it was recorded.

Mueller Hinton Agar (LOT NO: 0000333943, Himedia Laboratory put. Ltd.) Medium was prepared by dissolving 38g of MHA in 100ml of distilled water and weighed into conical flask and was covered with aluminum foil paper. It was autoclave at 121°C for 15 mins. After the agar has boiled into complete dissolutions and allowed to solidify at 37°C. Each bacterial strain were spread on the prepared agar using swab stick and allowed it to dry for 10 mins. The paper disc which was soaked with the extract and the control was placed on the Mueller Hinton Agar plate.

2.3. Statistical Analysis

Statistical analysis was done using the Software Statistical Package for Sciences (SPSS) for data originated. The data were interpreted based on the result of no inhibition zone of diameter (mm) of the extracts and to indicate the statistical significant differences. The values of the Minimum Inhibitory Concentration (MIC) at different concentrations of ginger and garlic extracts which is positive against the bacterial strains used were entered in the SPSS Software for statistical analysis. The data were analyzed using one-way analysis variance (ANOVA) and Turkey post-hoc test was used for comparison within the group and with different groups. The results was presented in tables. Statistical significance level was established at P 0.05.

3. Results

3.1. Minimum inhibitory concentration results

Table 1 Antibacterial activity of ethanol, n-hexane, ethanol + water, aqueous plant extract of ginger against *Escherichia coli*

Concentration (µm/g)	EE	NHEX	E + W	AQUE
25	32	28	29	33
20	29	22	23	28
15	26	20	20	25
10	20	18	18	19
5	16	13	11	14

Keys: EE means Ethanol, NHEX means N-hexane, E + W means Ethanol + Water, AQUE means Aqueous

Table 2 Antibacterial activity of ethanol, n-hexane, ethanol + water, aqueous plant extract of ginger against *Salmonella spp*.

Concentration (µm/g)	EE	NHEX	E + W	AQUE
25	30	30	31	35
20	24	26	26	30
15	22	25	24	27
10	16	17	19	25
5	13	15	14	20

Keys: EE means Ethanol, NHEX means N-hexane, E + W means Ethanol + Water, AQUE means Aqueous

Table 3 Antibacterial activity of ethanol, n-hexane, ethanol + water, aqueous plant extract of garlic against *Escherichia coli.*

Concentration (µm/g)	EE	NHEX	E + W	AQUE
25	30	27	26	31
20	27	24	23	29
15	23	20	19	26
10	18	16	14	22
5	13	12	11	19

Keys: EE means Ethanol, NHEX means N-hexane, E + W means Ethanol + Water, AQUE means Aqueous

Table 4 Antibacterial activity of ethanol, n-hexane, ethanol + water, aqueous plant extract of garlic against *Salmonella spp.*

Concentration (µm/g)	EE	NHEX	E + W	AQUE
25	30	27	30	30
20	25	24	27	26
15	22	20	24	25
10	19	16	20	17
5	15	12	16	15

Keys: EE means Ethanol, NHEX means N-hexane, E + W means Ethanol + Water, AQUE means Aqueous

3.2. Statistical Analysis Result

Table 5 The mean inhibition zone of ginger and garlic against *Escherichia coli*

Conc.(µm/g)	EE	E+W	N-Hexane	Aqueous
25	10.667 ± 10.000a	9.667±8.667a	9.333±9.000a	11.000±10.333a
20	9.667±9.000a	7.667±7.667a	7.333±8.000a	9.333±9.667a
15	8.667±7.667a	6.667±6.333a	6.667±6.667a	8.333±8.667a
10	6.667±6.000a	6.000±4.667a	6.000±5.333a	6.333±7.333a
5	5.333±4.333a	3.667±3.667a	4.333±4.000a	4.667±6.333a
Total	7.800	6.467	6.667	8.200

Legend: EE means Ethanol, E + W means Ethanol + Water, Conc. means concentration

Table 6 The mean inhibition zone of ginger and garlic against *Salmonella spp*.

Conc.(µm/g)	EE	E+W	N-Hexane	Aqueous
25	10.000 ± 10.000a	10.333±8.667a	10.000±10.000a	11.667±11.000a
20	8.000±8.333a	8.667±7.667a	8.667±9.000a	10.000±9.667a
15	7.333±7.333a	8.000±6.333a	8.333±8.000a	9.000±8.333a
10	5.333±6.333a	6.333±4.667a	5.667±6.667a	8.333±7.000a
5	4.333±5.000a	4.667±3.667a	5.000±5.333a	6.667±6.000a
Total	7.200	7.100	7.667	8.767

Legend: EE means Ethanol, E + W means Ethanol + Water, Conc. means concentration

4. Discussion

In the present study, antibacterial effect of ginger extracts was evaluated by disc diffusion method using different plants extracts. The extracts used were ethanol, n-hexane, ethanol + water, aqueous. The comparative investigation was conducted between selected plants ginger and garlic for antibacterial activity. Ginger extract did not show any inhibitory effect against the test microorganisms. In other words, all tested bacteria strains showed poor susceptibility to the ginger aqueous extracts in the study [9]. In this study, the various concentrations of ginger and garlic failed to inhibit the growth of all the test microorganisms. The lowest concentration did not permit any visible growth when compared with the control was considered to be minimum inhibitory concentration. Ginger and garlic extracts did not showed antibacterial activity at all concentration (25, 20, 15, 10 and 5) to all test microorganisms in the present of investigation. Ginger extract was also found to have no antibacterial properties against *E. coli*, *Salmonella spp.* Furthermore, the comparison of the inhibitory activity of ginger and garlic extracts with antibiotics revealed that chloramphenicol had the highest zone of inhibition. Ginger is effectives in the treatment of human ill-health conditions such as vomiting, nausea [10].

The antibacterial activity of the ethanolic extracts of garlic and ginger against some enteric bacteria was reported; however ginger seems to be ineffective against organism as observed in our study. The findings of this study have many importance on both ginger and garlic and they should be widely cultivated for their medicinal uses. The pharmacological activities on ginger include antimicrobial, antibacterial etc [11]. Ginger enhances resistance to infectious disease which increase specific and non- specific mechanism [12]. Garlic (*Allium sativum*), is the other closely related species including the shallot, the onion and others. Garlic has biologically active compounds like sulfur containing compounds (Allicin and Diallylsulfides) that act as antimicrobial effects against many viruses, bacteria, fungi, parasites and antioxidant, antithrombotic and vasodilator characteristics. It has several synergistic effects that either prevent or possibly may fight cancer. The action of garlic has been attributed to stimulate immune effector cells including T-cell and natural killer cells. Nausea and vomiting are other major adverse effects and care should be taken in consuming high quantities Garlic reduces the risk of patients with prostate cancer especially those with localized disease [13].

5. Conclusion

In conclusion, this study has shown that ginger and garlic possess medicinal properties has they have fight infectious diseases. The use of ginger has shown to be effective in treating diseases in humans, poultry and aquaculture owing to its antimicrobial, antioxidant, growth promoter and immune-stimulant properties. Clearly more studies are needed to refine the use and improvement of the efficacy of this important medicinal plant. Further work needs to be done to identify the chemical nature of the active principles as well as their roles in diseases curing.

Compliance with ethical standards

Disclosure of conflict of interest

All the authors hereby declared that there is no conflict of interests whatsoever.

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