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Antimicrobial Effects of Garlic and Ginger Plants Extracts on Certain Clinical Sample

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ABSTRACT

The antibacterial properties of n-hexane and methanol extracts from ginger and garlic were evaluated in vitro against Klebsiella pneumoniae, Escherichia coli, Salmonella enterica,, and Shigella dysenteriae using the agar well diffusion method. Phytochemical analysis indicated the presence of flavonoids, saponins, carbohydrates, alkaloids, and triterpenes in the extracts. The garlic extracts, both n-hexane and methanol, demonstrated the highest effectiveness against S. dysenteriae, achieving maximum inhibition zones of 27 mm at 40 mg/ml and 29 mm at 80 mg/ml. In contrast, the ginger extracts were more effective against E. coli, with maximum inhibition zones of 16 mm at 40 mg/ml and 19 mm at 80 mg/ml. The minimum inhibitory concentration (MIC) of the methanol extract for the tested organisms ranged from 5 to 20 mg/ml, while the n-hexane extract showed MIC values between 2.5 and 10 mg/ml. The methanol extract of ginger had MIC values ranging from 10 to 40 mg/ml, and the n-hexane extract had values between 10 and 20 mg/ml. The minimum bactericidal concentration (MBC) of the methanol garlic extract was between 10 and 40 mg/ml, while the n-hexane garlic extract had MBC values ranging from 2.5 to 20 mg/ml. The methanol ginger extract's MBC values were between 10 and 40 mg/ml, and the n-hexane ginger extract had MBC values between 10 and 20 mg/ml. The findings of this study indicate that these extracts possess antibacterial activity against the tested organisms, suggesting their potential for drug development.

Keywords: Antimicrobial effects, garlic extract, ginger extract, n-hexane extract, methanol extract

INTRODUCTION

Ginger, scientifically known as Zingiber officinale (referred to as ‘jinja’ in Igbo, ‘cithar’ in Hausa, and ‘Atale’ in Yoruba), is a tall, slender herbaceous perennial that features a thick, fleshy underground rhizome and one or more leafy stems that can reach heights of up to 1.25 meters. This plant thrives in the tropical climates of Australia, West Africa, India, Jamaica, Brazil, China, and certain regions of the United States (Suruchi et al., 2016). In its first year, ginger produces a straight green stalk approximately 60 cm tall, which emerges from the

rhizome. Its leaves, measuring between 12 and 30 cm in length, wither each year. Ginger prefers warm, sunny environments and can benefit from some shade during hot weather, particularly when it is young, although shading is generally seen as unnecessary. The ideal rainfall for ginger cultivation is between 2500 and 3000 mm, evenly distributed throughout the year. The plant is known for its distinctive, aromatic, and pungent scent and flavor (Shubha, 2015).

Fresh, powdered, and preserved ginger are commonly used as spices. Fresh ginger is versatile in cooking, as well as in beverages like ginger ale. Ground ginger is primarily used in culinary applications and can also serve as a flavoring in processed foods. Conversely, preserved ginger is utilized in the production of processed items such as jams, marmalades, cakes, and candies (Sharifi-Rad et al., 2017). For centuries, ginger has been valued as both a spice and a medicinal herb in China and India.Ginger plants were cultivated in pots and transported on lengthy sea journeys to help prevent scurvy (Suruchi et al., 2016). The oil derived from ginger possesses antimicrobial properties due to its components, which include eugenol, thymol, 1,8-cineole, pinenes, linalool, and terpineol (Suruchi et al., 2018). Fresh ginger is utilized in treating various ailments such as cold-related illnesses, nausea, asthma, cough, colic, heart palpitations, swelling, dyspepsia, loss of appetite, and rheumatism. For asthma and cough relief, fresh ginger juice can be combined with a small amount of fresh lemon juice and honey (Ponmurugan and Rajaram, 2018). Ginger is significant in medicine because of its active constituents, including gingerol, paradol, shogoal, zingerone, zerumbone, terpenoids, and ginger flavonoids (Arshad et al., 2019).

Allium sativum, known as ‘aayu’ in Yoruba, ‘ayo-ishi’ in Igbo, and ‘tafarunua’ in Hausa, is a perennial bulbous plant that originated in Central Asia and is now cultivated worldwide. Garlic can reach heights of up to 2 feet or more. The bulb is the primary medicinal part of the plant (Steven, 2015). Each garlic bulb consists of 4 to 20 cloves, with each clove weighing approximately 1 gram. Garlic can be used fresh, aged, dried, or in supplement form, with each type potentially having different effects on the body (Sethi et al., 2014). It is widely used as a seasoning and helps prevent certain heart diseases, including atherosclerosis.

High cholesterol, high blood pressure, and enhanced immune function, along with cancer protection, are benefits associated with garlic (Steven, 2015). The medicinal properties of garlic are attributed to its content of glycosides, vitamins B, C, and D, as well as allisatin I and II. Additionally, it contains volatile sulfur oil, which has a worm-expelling effect (Arshad et al., 2019).

` The antimicrobial effects of garlic and ginger can be tested against Escherichia coli, Salmonella enterica, Klebsiella pneumoniae, and Shigella dysenteriae. These microorganisms are sensitive to even minor changes in their environment; they tend to proliferate rapidly in favorable conditions but decrease in number when conditions become unfavorable. The issue of microbial resistance to antimicrobials is a persistent global challenge. However, plant-derived antimicrobials typically have fewer side effects compared to many synthetic antimicrobial agents (Ehigbai et al., 2016). This study was conducted to assess the antimicrobial effects of garlic and ginger on Escherichia coli, Salmonella enterica, Klebsiella pneumoniae, and Shigella dysenteriae.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The plant materials were sourced from Umuaka market in Njaba LocalGovernmentArea, Imo State, Nigeria. An Botanist from the Department of Plant Biology at Imo state University (IMSU) identified them as ginger and garlic

Extracts preparations

The ginger was thoroughly washed with distilled water to remove any sand and then air-dried at room temperature for six weeks. The garlic bulbs were divided into cloves, with the skins removed. The cloves were sliced and air-dried at room temperature for approximately seven weeks. The dried materials were then ground using a sterile laboratory mortar and a sterile electric blender to create a uniform sample. Each powdered sample (120 g) was extracted with 750 ml of methanol and n-Hexane using the cold maceration method as outlined by Handa et al. (2018). After extraction, the plant materials were concentrated using a rotary evaporator at 40°C. The resulting extract was freeze-dried to eliminate some of the water content. It was then stored in sterile sample bottles and kept in a refrigerator at 4°C until it was needed for further analysis. Portions of the plant extracts were subsequently subjected to phytochemical screening.

Screening of phytochemicals

The phytochemical screening of the plant extracts for secondary metabolites was conducted following the method outlined by Harborne (2019). This screening aimed to verify the presence or absence of specific phytochemical compounds known to contribute to the antimicrobial properties of the plant extracts, including triterpenes, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins, and alkaloids.

Test Organisms collection

Clinical isolates of Escherichia sp., Salmonella sp., Klebsiella sp., and Shigella sp. were collected in broth medium from patients at Old General Hospital in Minna, Niger State, Nigeria, and transported to the laboratory in ice packs. The isolates were confirmed by subculturing on MacConkey agar (MCA), Eosin Methylene Blue (EMB), and Salmonella-Shigella agar (SSA). The resulting colonies were then Gram stained and subjected to various biochemical tests, including the Catalase Test, Methyl Red-Voges Proskauer Test, Indole Test, Simmon's Citrate Test, Oxidase Test, Urease Test, and Triple Sugar Iron Agar (TSIA) (Clarke and Cowan, 2019; Daniel, 2019). The pure isolates were preserved in Nutrient agar slant bottles at a temperature of 4°C until needed for further experimentation.

Antibacterial Assay

The agar well diffusion method was used to evaluate the antibacterial activity of the plant extracts, following the guidelines set by the Clinical and Laboratory Standards Institute (CLSI) (2019). The standardized suspension was applied to the surfaces of sterile nutrient agar plates using a sterile cotton swab. Sterile core borers were used to create 8 mm diameter wells in the solidified agar. Each well was sealed with 1 ml of sterile nutrient agar. The wells were then filled with the specified concentrations of the plant extracts (40 mg/ml and 80 mg/ml). The plates were allowed to sit at room temperature for approximately 3 hours to facilitate diffusion of the extracts, after which they were incubated at 37°C for 24 hours. The antibacterial activity of the plant extracts was assessed by observing the formation of zones of inhibition around the wells, while the absence of such zones indicated a lack of antibacterial activity.The antibacterial properties of the plant extracts were evaluated against amoxicillin (0.25µg/ml) to assess their

effectiveness. The control plates included an extract sterility control (ESC), a medium sterility control (MSC), and an organism viability control (OVC).

Assessment of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration was determined following the CLSI guidelines (2019). The plant extract was dissolved in 5 ml of solution (0.5 ml DMSO and 4.5 ml water). Two milliliters of sterile nutrient broth were added to five different test tubes, followed by the addition of 2 ml of various concentrations of the extract. The test organism was then inoculated into the labeled tubes, excluding the negative control. The tubes were incubated at 37°C for 24 hours. This process was repeated for the other extracts and test organisms. The MIC was defined as the lowest concentration that inhibited any visible growth.

Assessment of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration was also determined according to CLSI guidelines (2019). The test tube that exhibited no visible growth was subcultured onto sterile nutrient agar and incubated at 37°C for 24 hours. The minimum concentration at which the organism did not grow was recorded as the minimum bactericidal concentration.

RESULTS AND DISCUSSION

Phytochemical Components of garlic and ginger Plants

The garlic extract produced a golden-yellow, sticky residue with a strong, unpleasant odor, while the ginger extract was brown and had a spicy-sweet aroma. The findings from the initial phytochemical screening of the n-Hexane and methanolic extracts of ginger and garlic are summarized in Table 1. The ginger extracts contained flavonoids, saponins, and triterpenes. However, the methanol extract of ginger included carbohydrates, which were not found in the n-Hexane extract. Both n-Hexane and methanolic extracts of ginger lacked phenols, tannins, alkaloids, cardiac glycosides, and steroids. In contrast, the n-Hexane and methanolic extracts of garlic contained flavonoids, saponins, alkaloids, and triterpenes. Carbohydrates were present in the methanolic extract of garlic but absent in the n-Hexane extract. Similarly, phenols, tannins, steroids, and cardiac glycosides were not detected in either extract. Overall, phenols, tannins, steroids, and cardiac glycosides were absent in all four extracts.

Table-1. Preliminary qualitative phytochemical analysis of n-Hexane and Methanol extracts of garlic and ginger extracts.

The organisms tested showed varying susceptibility to different concentrations of the plant extracts. The antibacterial properties of methanol and n-hexane extracts from ginger and garlic are linked to the presence of bioactive compounds (Ponmurugan and Rajaram, 2019; Dixon and Jeena, 2017). The extracts contained flavonoids, carbohydrates, saponins, triterpenes, and alkaloids, which align with findings from earlier research (Cheeke, 2019; Abdullahi et al., 2019; Aliyu et al., 2017). However, phenols, tannins, steroids, and cardiac glycosides were not detected in any of the four extracts.

Garlic extracts produced the largest zones of inhibition compared to ginger extracts against all bacterial isolates. This antibacterial activity may be due to the secondary metabolites

present in these plants (Patra and Saxena, 2019). It was also noted that the extraction solvent influenced the sensitivity of the test organisms, as mentioned by Abdullahi et al. (2019). While the antibacterial activity of ginger extracts was generally low, Aliyu et al. (2017) found that ginger extract showed stronger antibacterial effects against various bacteria, although the mixed results may be due to differences in ginger preparations and concentrations. The reduced activity of ginger extracts could also stem from the possibility that the active components did not exhibit effectiveness in vitro against the clinical isolates, or that the concentrations used were insufficient.The concentrations were not sufficient to produce antimicrobial effects against the organisms, as noted by Cheeke (2019) and Aliyu et al. (2017).

A high minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) indicate low antimicrobial activity, while low MIC and MBC values suggest high activity of the plant material. In this study, garlic extracts exhibited lower MIC values compared to ginger extracts when tested against the bacterial isolates. Notably, the n-hexane garlic extract had the lowest MIC value of 2.5 mg/ml against Klebsiella sp., while the methanolic garlic extract showed the highest MIC value of 20 mg/ml against Salmonella sp. Conversely, the methanolic ginger extract also had a high MIC value of 40 mg/ml against Salmonella sp., with the lowest MIC value for this extract being 10 mg/ml These findings align with the results reported by Iram et al. (2019) and Ponmurugan and Rajaram (2019).

Both extracts exhibited the same minimum bactericidal concentration (MBC) values for two of the isolates. The methanolic and n-hexane garlic extracts showed MBC values of 40 and 20 mg/ml, respectively, against Salmonella sp., while the methanolic and n-hexane ginger extracts had identical values against Shigella sp. The garlic extracts were effective in killing Shigella sp. and Klebsiella sp., and the ginger extracts also killed Salmonella sp. and Klebsiella sp., although only the n-hexane ginger extract was effective against Escherichia coli. When comparing the MBC values of garlic extracts to those of ginger extracts, garlic demonstrated significantly higher activity in vitro against the tested organisms, as noted by Aliyu et al. (2019). Additionally, the n-hexane extracts were found to be more effective than the methanolic extracts, which contradicts the findings of Garba et al. (2019). This highlights the influence of the solvent system, which significantly impacts the antibacterial properties of the crude extracts.

CONCLUSION

This work demonstrated that S. dysenteriae, S. enterica, E. coli, and K. pneumoniae were sensitive to the extracts of ginger and garlic. This indicates that these plants possess antibacterial properties and could serve as a potential source of active antimicrobial agents for developing treatments against these infectious microorganisms.

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