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Antimicrobial Activities of Oyster Mushroom (*Plerotous ostreatus*), Garlic (*Allium sativum*), and Ginger Extracts (*Zingiber officinale*) Against Some Clinical Isolates

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Abstract

The prevalence of multiple drug resistance among human pathogenic bacteria necessitates a continual search for new antimicrobial medicines, particularly among plants that are frequently farmed or naturally found in our surroundings. The study aims to investigate the inhibitory effect of garlic, ginger, and mushroom extracts at different concentrations against some clinical isolates. Anti-bacterial components from mushrooms, garlic, and ginger were extracted with hot water, cold water, ethanol, and acetone, and their anti-bacterial activity was determined using the agar well diffusion method. Zones of inhibition were observed primarily on hot water extracts of mushrooms (*Pleurotus ostreatus*) on culture plates inoculated with *Staphylococcus aureus, Escherichia coli*, and *Klebsiella pneumonia* at 37^0C for 24 hours. The cold water extracts of the mushroom (*Pleurotus ostreatus*) gave the highest zone of inhibition of 14.0±1.0mm when used against *S. aureus*. For spices, the cold water extracts yielded the highest zones of inhibition of 22.0±1.0mm followed by 16.0±1.0mm as observed with ginger. The results obtained have shown clearly that the mushrooms (*Pleurotus ostreatus*), garlic (*Allium sativum*), and ginger (*Zingiber officinale*) extracts contain phytochemicals with some antimicrobial activities. The water extracts of the mushrooms and spices showed broad-spectrum antimicrobial activity much more than ethanol and acetone extracts. The antimicrobial activities of mushroom and garlic extracts were highly effective against the bacterial pathogens studied. However, the antimicrobial activity of the ginger extract was poor. To address the multi-drug resistance to antibiotics, I recommend÷ that bioactive compounds found in mushrooms, ginger, and garlic be patented and used as alternative antimicrobials.

Keywords: Antimicrobial agents; Clinical isolates; Drug resistance; Mushrooms; Garlic; Ginger.

INTRODUCTION

Traditional medicine has historically used a variety of medicinal plants to treat drug-resistant disorders (Curto et al., 2021). As a result, infectious diseases remain a significant concern for human health (Frieri et al., 2017). Therefore, the continued expansion of multidrug-resistant bacteria, and rising antibiotic overuse, underscore the need for alternate agents. Many communities have long utilized herbs and spices to treat various diseases, including antimicrobials (Dog, 2006; Leja & Czaczyk, 2016; Dini, 2018). Herbs and spices, unlike conventional antibiotics, are typically regarded as safe for humans due to their long history of use in food preparation (Akullo et al., 2022). These plants are classified as natural products and represent an important source of innovative natural medications. The prevalence of multiple drug resistance among human pathogenic bacteria necessitates a continual search for new antimicrobial medicines, particularly among plants that are frequently farmed or naturally found in our surroundings (Anyamaobi et al., 2020).

Furthermore, studying the dietary traditions of various ethnic groups worldwide has significantly impacted the development of research into natural products such as mushrooms, garlic, and ginger. Their use as an alternative treatment for numerous diseases has over during the last few decades. (Vuorelaa et al. 2004; Ansari et al. 2006). Herbs and spices cause fewer adverse effects than manufactured medications. They are affordable, have greater patient tolerance, and are widely available to low-income people (Adeshina et al., 2011; Emmanuel et al., 2021). This has led to the discovery of a link between the chemical structure of molecules found in natural products, their biological properties, and their significance to human health (Emmanuel et al., 2021).

However, most of these natural chemicals with antibacterial potential have yet to be validated in terms of their purported effects, albeit medicines based on them may be created (Goncagul et al., 2009). Several studies have been published on herbs and spices' antibacterial and antifungal activities. Still, nothing is known regarding the precise mechanism of their antimicrobial action (Gur et al., 2006; Pattaratanawadee et al., 2006; Yusha'u et al., 2008; Belguith et al., 2010; Poeloengan, 2011; Gull et al., 2012). Ginger (*Zingiber officinale*) is widely distributed across the tropics of Asia, Africa, America, and Australia, where it is used as a spice and medicinal plant (Jung & Pezzuto, 2002). Numerous investigations have revealed the antibacterial properties of ginger and garlic (Akintobi et al., 2013; Indu et al., 2006; Khashan, 2014; Mohammed et al., 2019). Since ancient times, Mushrooms have been considered delicacies high in proteins, vitamins, minerals, and fiber. Furthermore, mushrooms have been extensively utilized in traditional medicine and are effective antibacterial agents (Kabelik, 2018). The medicinal activities of mushrooms are attributable to various bioactive biomolecules such as polysaccharides, high and low molecular weight compounds, glycoproteins, and immune-modulating (Bolzani et al., 2006). The fruiting bodies and mycelium of mushrooms exhibit health-promoting values such as immuno-stimulatory, antibacterial, and anti-oxidative properties (Gull et al., 2012). The simultaneous impact of these medications would have beneficial therapeutic effects. However, most of these natural products, which have indicated antimicrobial potentials, have yet to be validated for their claimed activities and possibly develop drugs from them (Goncagul et al., 2009).

Ginger (*Zingiber officinale*) Ginger, a rhizome that is white to yellow and very aromatic, has lots of beneficial uses in medicine for the treatment of high blood pressure, cold-induced diseases, asthma, persistent cough, and other related problems of the respiratory system, and in the flavoring of foods (Kumar et al., 2011). There is a broad spectrum of antibacterial activity of ginger rhizome against a range of Gram-negative and Gram-positive bacteria (Mohammed et al., 2019). Despite this, there are different opinions about ginger's antimicrobial properties against microorganisms from various sources (Abdallah & Abdallah, 2018).

Garlic is an onion with a distinctive spicy, pungent flavor that has long been utilized for culinary and medicinal purposes. Garlic is an antioxidant and can help treat the common cold and cardiovascular problems. In addition, Garlic's antimicrobial activities are linked to some bioactive compounds (Tsao & Yin, 2001). Because infectious diseases have a high morbidity and mortality burden, many studies have been conducted better to understand their control, treatment, and prevention (Murugaiyan et al., 2022). When some edible mushrooms, garlic, and ginger come into touch with them, they either suppress or kill various microbes. Some examples include S. *aureus, E. coli, B. cereus,* and *K. pneumoniae*. This current work, therefore, evaluated the antibacterial properties of extracts of edible mushrooms (oyster mushroom) and two spices (ginger and garlic) against *Staphylococcus* spp., *E. coli*, and *Klebsiella* spp.

The study's objective is to investigate the inhibitory effect of garlic, ginger, and mushroom extracts at different concentrations against some clinical isolates, and determine the sensitivity of pathogenic organisms to various antimicrobial compounds as treatment options for patients.

MATERIALS AND METHOD

Materials

Mushroom, Ginger, Garlic, Nutrient agar, Blood agar, Mueller Hinton Agar, wire loop, forceps, bijou bottles, distilled water, stainless steel cork borer, test tubes, aluminum foil, test tube rack, filter papers, sterile cotton swab sticks, cotton wool, hand gloves, marker, nose mask, weighing balance.

Sample collection

Garlic (Figure 1) and Ginger (Figure 2) were bought from the Ore market, Odigbo Local Government in Ondo state, Nigeria. It was placed in sterile Polyethylene bags and kept at a temperature of 4 ºC until use and *Pleurotus ostreatus* (Figure 3) (Was collected from Odo - Lawe Farm at Ode- Erinje, Okitipupa Local Government in Ondo State). The materials were brought to the laboratory and preserved at room temperature. All practical work was done at the Medical Microbiology Laboratory of State Specialist Hospital, Okitipupa, Ondo State. Mr. David, a Medical Laboratory Technician (MLT) at State Specialist Hospital, Okitipupa authenticated the samples.

Test Organisms

Pure *E. coli*, *S. aureus*, and *K. pneumoniae* cultures were taken at the State Specialist Hospital's Medical Microbiology Laboratory in Okitipupa, Ondo State, Nigeria. The cultures were sub-cultured to resuscitate them in the laboratory and kept at 4º C as stock culture.

Figure 1. Dried Pleurotus *ostreatus.*

Figure 2. *Allium Sativum.*

Figure 3. *Zinigiber officinale.*

Extraction of Mushrooms, Ginger and Garlic

The extraction was performed using Faqir and Farhan's procedure (2011). The respective mushroom, ginger, and garlic samples were cut into bits, sun-dried for 6 days, and then pulverized into powder using an electric grinder. 20 g each of the pulverized mushroom, ginger, and garlic were weighed and soaked in 200 ml of cold water, hot water, ethanol, and acetone, respectively. It was allowed to stand for 7 days only with interval shaking at 30 minutes (this is to prevent mold from growing in the extraction water). The filtrates were poured into different well-labeled beakers and evaporated at 37°C for 72 hours using a water bath. The extracts were collected and a 10 mg/ml stock solution was produced. Stock solutions were generated at 10 mg/ml concentrations, and different concentrations were created by serially diluting the stock solution.

Sensitivity Test

Mueller Hinton Agar and nutrient agar plates were prepared using Kirby Bauer's method. Fresh inoculums of the test organisms were added to the media using the pour-plate method. The Control was determined using pure solvent instead of the extracts. The plates were incubated at 37 ^{0}C for 24 hours in the incubator, and the zone of inhibition in diameter was measured using the nearest millimeter (mm), as shown in plate 1.

Statistical analysis

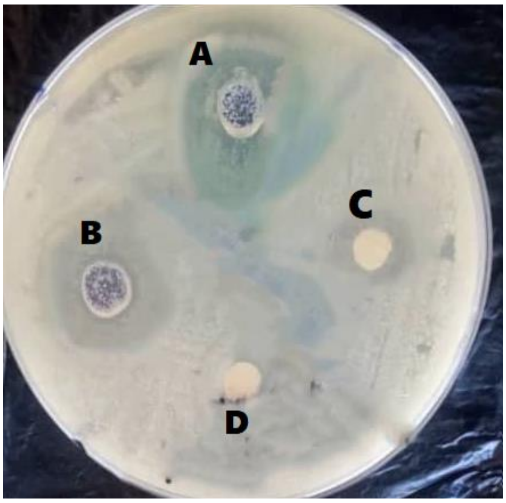
Quantitative data was reported as mean ± SD. The Data obtained were analyzed using SAS's variance analysis (ANOVA) procedure of SAS (Statistical Analysis Software).

RESULTS AND DISCUSSION

Results

The inhibitory effect of Mushroom, Garlic, and Ginger extracts

As shown in Table 2, the formal antibiotic used displayed a pronounced inhibitory effect against *E. coli* with a 30.0 mm diameter as the zone of inhibition among all the tested organisms. at the same time, the result revealed that ethanolic extracts of ginger showed the highest antimicrobial activity when used against *K. pneumoniae* with a zone of inhibition diameter of 26.0 mm and most minor activity when used against *S. aureus* and *E. coli* with a zone of inhibition diameter of 17.0 mm and 15.0 mm respectively. The result of activity in this study is similar to previous reports by Gull et al. (2012), Riaz et al. (2015), and Anyamaobi et al. (2020). Acetone extract from mushrooms showed the highest inhibition against *E. coli* with a zone of inhibition diameter of 24.0 mm~~,~~ ; no antimicrobial activity was observed against *S. aureus* and *K. pneumoniae* . This is in agrees with the result of Adeshina et al. (2011), which showed high susceptibility of *Escherichia coli* but no activity against *Staphylococcus aureus*. However, garlic showed the highest antimicrobial activity against *S. aureus*, with a zone of inhibition diameter of 16.0 mm and minor activity against *E. coli* and *K. pneumoniae*, each producing a zone of inhibition of 11.0 mm. In comparison, ginger showed the highest antimicrobial activity against *E.* coli with a zone of inhibition diameter of 16.0 mm and most minor activity against *S. aureus* and *K. pneumoniae* with *a* zone of inhibition diameter of 13.0 mm and 11.0 mm, respectively. On the other hand, hot water extract of mushrooms showed the highest antimicrobial activity against *S. aureus* with a zone of inhibition diameter of 30.0 mm and minor activity when used against *E. coli* with a zone of inhibition diameter of 10.0 mm. At the same time, there was no zone of inhibition against *K. pneumonia*. However, hot water garlic extract showed the highest antimicrobial activity when used against *S. aureus* with a zone of inhibition diameter of 25.0 mm and minor activity when used against *E. coli* with a zone of inhibition diameter of 13.0 mm with no zone of inhibition against *K. pneumoniae*. Results also revealed that cold water extracts of garlic showed the highest inhibition when used against *S. aureus* with a zone of inhibition diameter of 22.0 mm and minor activity when used against *E. coli* and *K. pneumoniae* with a zone of inhibition diameter of 11.0 mm and 13.0 mm, respectively. However, mushrooms showed the highest antimicrobial activity when used against *S. aureus* with a zone of inhibition diameter 14. 0 mm and most minor activity when used against *E. coli* and *K. pneumoniae,* each producing zone of inhibitions diameter 10.0 mm. Details of the results can be seen in Table 1 below. For the minimum inhibitory concentration, all the tested organisms could grow at the concentration of 10 and 20 mg/ ml of *Pleurotus ostreatus*, *Allium sativum, and Zingiber officinale,* as shown in Table 3. None of the tested isolates could grow at 100 mg/ml concentrations. Also, all test isolates except *K. pneumoniae* grew at a concentration of 60 mg/ml.



Key: A- Ethanol extract of garlic; B- Cold water extract of garlic; C- Hot water extract of garlic; D-Pure Solvent

Figure 1. Minimum inhibitory concentration of garlic extract bacterial isolate.

Table 1. Minimum inhibitory Zones of extracts of mushroom, ginger, and garlic against test organisms using agar well diffusion method.

Samples	Solvent	*S. aureus*	*E. coli*	*K. pneumniae*	control
Pleurotous ostreatus	Ethanol	11.0±0.6	11.0±I.5	-	0.00
	Acetone	-	24.0±1.0	-	0.00
	Hot water	30.0±1.0	11.0±1.0	-	0.00
	Cold water	14.0±1.0	-	-	0.00
Allium sativum	Ethanol	15.0±1.0	11.0±1.5	24.0±1.0	0.00
	Acetone	15.0±0.7	11.0±1 .5	11.0±1.5	0.00
	Hot water	25.0±1.5	13.0±1.0	-	0.00
	Cold water	22.0±1.0	11.0±1.5	13.0±1.0	0.00
Zingiber Officinale	Ethanol	17.0±0.7	15.0±1.0	26.0±1.0	0.00
	Acetone	13.0±1.0	16.0±0.5	11.0±1.5	0.00
	Hot water	13.0±.01.0	-	11.0±1.5	0.00
	Cold water	16.0±1.0	11.0±1.5	11.0±1.5	0.00

Table 2. Inhibition zones of standard antibiotics (mm) against test organisms.

Isolates	Ciprofloxacin	Augmentin	Cephalexin	Gentamycin
S. aureus	15.0±0.3	20.0±0.6	15.0±0.3	25.0±0.3
E. coli	20.0±0.6	30.0±0.3	7.01±0.5	30.0±0.3
K. pneumoniae	20.0±0.6	20.0±0.6	7.01±0.5	0.0±0.00

Table 3. Minimum Inhibitory Concentration of mushroom, garlic, and ginger extracts at different concentrations.

Samples	Solvent	100 mg/ml	60 mg/ml	20 mg/ml	10 mg/ml
Pleurotous ostreatus	*S. aureus*	-	+	+	+
	E. coli	-	-	+	+
	K. pneumoniae	-	-	+	+
Allium sativum	*S. aureus*	-	+	+	+
	E. coli	-	+	+	+
	K. pneumonia	-	-	+	+
Zingiber officinale	*S. aureus*	-	+	+	+
	E. coli	-	+	+	+
	K. pneumonia	-	-	+	+

Key: - Means no growth, + Means there is growth.

Discussion

In this study, garlic extract showed a wide inhibition zone, followed by combining garlic, mushroom, and ginger extract. The results obtained in this study explained the relatively higher therapeutic efficacy of the plant materials on both Gram-positive and Gram-negative bacteria. The antimicrobial properties of garlic primarily rely on the presence of allicin, a thiosulfate compound found in crushed garlic bulbs (Wallock-Richards et al., 2014), as well as antibacterial sulfur bio-compounds like alliin and alliinase (Mardomi, 2017; Singh, 2018). On the other hand, ginger's antimicrobial activity can be attributed to its essential oil or oleoresins (Beristain-Bauza et al., 2019), as well as phenolic compounds such as eugenol, shogaols, Zingerone, gingerdiols, and gingerols interact synergistically with other chemicals such as β-sesquiphellandrene, cis-caryophyllene, zingiberene, α-farnesene, and α- and β-bisabolene (Mardomi, 2017; Mara-Teles et al., 2020). As a result, there is an urgent need for developing new and effective medications against present antibiotic-resistant bacteria. Baulori et al. (2016). This study investigated the antibacterial activity of mushrooms, garlic, and ginger using agar well diffusion. The findings suggested that extracts from mushrooms, garlic, and ginger have antibacterial capabilities, as Fasidi (2010) reported. These natural products have promising therapeutic use against human pathogens such as bacteria, fungi, and viruses. Variations in the degree of antimicrobial activity of mushrooms were also observed in this study. This result agrees with the report of Akyuz et al. (2010) and Jangadish et al. (2009). The broad spectrum of mushroom activity was also brought to light as the extracts of mushrooms showed an inhibitory effect on clinical isolates used for this investigation. These suggest that the bioactive products in mushrooms are in concentrations that exude varying degrees of antimicrobial activity. Furthermore, the study revealed that the bacterial isolates (*Staphylococcus aureus* and *Escherichia coli*) showed more sensitivity to mushroom extracts (Table 1). Also, extracts from garlic and ginger, as indicated by (Table 1), have a wide spectrum of antimicrobial activity with varying degrees of sensitivity when used against clinical isolates. However, the data from Table 1 revealed that extracts from garlic and ginger had antibacterial against all tested bacterial isolates except *K. pneumoni~~ia~~* in hot-water garlic extracts and *E. coli* in hot-water ginger extracts. This finding is by the findings of (Belguith, 2010; Gull, 2012). The results obtained in this study explained the relatively higher therapeutic efficacy of the plant materials on both Gram-positive and Gram-negative bacteria. However, gram-positive bacteria demonstrated more sensitivity than gram-negative bacteria. This is in collaboration with the findings of (Anyamaobi et al. 2020). The sensitivity of isolates to mushroom, garlic, and ginger extracts implies that the intrinsic substance in the extracts is unknown to the microorganisms, making it impossible to resist. Also, when the effects of ethanol, acetone, cold water, and hot water on mushrooms, garlic, and ginger are compared regarding inhibition of microbial growth, the result showed that the hot water and ethanolic extracts had greater inhibiting effects than acetone and cold water. Ethanol and hot water extracts of the antimicrobial compounds contained higher bioactive substances than cold water extract~~,~~; this is in collaboration with the findings of (Baulori et al., 2016). The result showed that the zone of inhibitions exhibited on agar plates by the mushroom, garlic, and ginger extracts were concentration-dependent. The higher the concentration of the extracts, the larger the zone of inhibition produced, as seen in plate 2 above. Numerous studies have been conducted with extracts of various plants, screening antimicrobial activity and discovery new antimicrobial compounds (Kabelik et al., *2018*). Therefore, medicinal plants are finding their way into pharmaceuticals and food supplements (Murugaiyan et al., 2022). However, it is important to note that the antimicrobial activity of garlic and ginger can vary depending on factors such as chemical composition, extraction solvent, methodology, and processing techniques (Singh, 2018). There are several advantages to using spices derived from plant origins as dietary supplements or alternative medicine, manifested by a reduction in the chances of developing antibiotic-resistant Bacteria emerging due to maintaining antibiotic use. Natural compounds, such as mushrooms and two spices, are among the most often used antimicrobial agents in food and have been utilized for thousands of years to treat various health issues, including infectious disorders. Besides, medical herbs may decrease the cost of treatment and minimize the development of adverse drug reactions. The garlic extract, ginger extract, mushroom extract, and their combination may prevent infection with pathogenic organisms and support their resistance against antibiotics.

CONCLUSION

Multi-drug-resistant organisms are becoming more common, posing a threat to treating an increasing number of infectious diseases. This study sheds more light on using plant-derived foods such as mushrooms, garlic, and ginger as alternative medicine or dietary supplements. This study concludes that mushrooms, garlic, and ginger contain significant antimicrobial compounds. More research is needed to investigate all of the antimicrobial compounds found in these natural products and their side effects. To address the issue of multi-drug resistance to antibiotics, I recommend that bioactive compounds found in mushrooms, ginger, and garlic be patented and used as alternative antimicrobials and also to focus more light on medicinal herbs to clarify their advantages and clinical applications to obtain low-cost treatment and prevents recurrent infections.

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