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Antimicrobial Efficacy of *Zingiber officinale* (Ginger) and *Allium sativa* (Garlic) Extract Against *Staphylococcus Aureus* Isolated From Urine Samples of Female Students Of Alex-Ekwueme Federal University Ndufu-Alike Ebonyi State

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Abstract

Methanol and ethanol extracts of garlic (*Allium sativa*) and ginger (*Zingiber officinale*) were investigated for their antimicrobial activities against *Staphylococcus aureus* isolated from urine samples of female students of Alex Ekwueme Federal University Ebonyi State using standard microbiology procedures. Out of fifty-four urine samples analyzed, thirty-two (64%) were positive for *Staphylococcus aureus* (64%). The antimicrobial activity of garlic plant extract was evaluated by the agar well diffusion method. The results show that ethanol extract from garlic and ginger plants was effective against *Staphylococcus aureus*. The inhibitory effect exerted by garlic extract was highest at the concentration of 100µg/ml with mean zone inhibition of $18 \pm 0.00\text{mm}$ which was statistically significant ($p < 0.05$) from the control $20.0 \pm 1.00\text{mm}$. Also, the inhibitory effect exerted by garlic was least at the concentration of 25µg/ml with a zone of inhibition of $10 \pm 0.0\text{mm}$ which was statistically significant ($p < 0.05$) from the control $20.0 \pm 1.00\text{mm}$. The activity of the methanol and ethanol extract was very minimal at low concentrations (25 µg/ml), but at higher concentrations (50-100 µg/ml), marked activity was observed. The minimum bactericidal concentration of both extracts was also determined and ranged from 25-100 µg/ml in both ethanol and methanol plant extract. The sensitivity of multiple drug-resistant organisms to these plant extracts implies an alternative or substitution for existing antibiotics. Therefore garlic and ginger extracts can be used as a source of antibiotic substances for possible treatment of *Staphylococcus aureus* infection still not preventing the use of antibiotics.

Keywords: Antimicrobial activity. Ginger, Garlic, Extraction solvent, *Staphylococcus aureus*.

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1.Introduction

Gram-positive *Staphylococcus aureus* is a cocci-shaped bacteria that is stained purple by the Gram stain and has a propensity to form clusters that are referred to as "grape-like." These organisms can flourish on media with up to 10% salt content, and their colonies frequently have golden or yellow hues. These organisms can grow aerobically or anaerobically (facultative) and at temperatures between 18oC-40oC. According to Rasigade and Vandenesch (2014), common biochemical identification tests include catalase positive (all pathogenic *Staphylococcus* species), coagulase-positive (to differentiate *Staphylococcus aureus* from other *Staphylococcus* species), novobiocin sensitive (to differentiate from *Staphylococcus saprophyticus*), and mannitol fermentation positive (to differentiate from *Staphylococcus epidermidis*). This opportunistic pathogen normally colonizes the human anterior nares. At the same time, this pathogen is one of the leading causes of life-threatening bloodstream infections, such as sepsis and endocarditis (Kwiecinski and Horswill, 2020). It is a major factor in the development of pneumonia and other respiratory tract infections, cardiovascular infections, infections at surgical sites, infections of prosthetic joints, and nosocomial bacteremia (Gordon and Micheal, 2021).

According to a review from 2012, there are 20 to 50 occurrences of *S. aureus* bacteremia per 100,000 people annually, and 10 to 30 percent of these patients will pass away as a result of the infection (Kourtis *et al.*, 2019). According to a more recent study from 2017 (Kourtis *et al.*, 2019), 20,000 Americans die each year from *S. aureus* bacteremia. Because of the myriad of virulence factors which include various toxins and enzymes, *Staphylococcus aureus* can cause a large diversity of both benign and fatal infections in humans and animals (Chen *et al.*, 2013). Its emergence has made it to be one of the most important human pathogens which is the leading cause of hospital and community-acquired infections (Chen *et al.*, 2013).

Ginger (*Zingiber officinale*), which belongs to the family Zingiberaceae and genus Zingiber, has been commonly consumed as a spice and as herbal medicine for a long time (Ayogu *et al.*, 2017). Ginger root is used to attenuate and treat several common diseases, such as headaches, colds, nausea, and emesis. Many bioactive compounds in ginger have been identified (Stoner, 2013). In recent times, ginger has been found to possess biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer (Citronberg *et al.*, 2013).

Garlic (*Allium sativum*) a member of the Amaryllidaceae family that is cultivated all over the world, provides noteworthy health benefits. Garlic was utilized as a medicine in many epidemics, including typhus, dysentery, cholera, and influenza about 1550 B.C. because antibiotics and pharmacy items were not yet available (Satiawan *et al.*, 2005). The therapeutic effects of garlic are mainly due to the impressive activity of its bioactive nature. Due to its medicinal benefits against cancer, cardiac disease, blood pressure, diabetes, bone and skin disorders, and other pathologies, thanks to its antioxidant, anti-inflammatory, and lipid-lowering activities, garlic consumption has recently attracted particular interest (Ansary *et al.*, 2020).

2. Materials and methods

2.1 Isolation of the organisms

The urine samples were first inoculated on nutrient broth and incubated at room temperature. The streaking method of culturing was employed for the isolation as described (Ani, *et al.*, 2015; Ayogu *et al.*, 2017). Growth on nutrient broth was subcultured on Mannitol salt agar plates, Eosin methylene blue plate, and incubated at 37°C for 24 hr.

2.2 Plant extracts preparation

Using a grinder, fully dried rhizomes were powdered. Exactly 1.0kg of the powdered samples were weighed and transferred to a paper cylinder placed into the soxhlet apparatus. It was soaked in methanol and ethanol for 24hr, followed by DMSO for 3 hr. The extraction was done by using the reflux method. Reflux is a process that involves condensing vapors and reintroducing the condensate to the system it originally came from. Water was supplied continuously to the condenser to cool the solvent, to prevent evaporation, and facilitate the process of extraction. The extract was distilled to remove the solvent to get a concentrated extract (Ephrem and Asefaw, 2016).

2.3 Antibacterial activity test

The antibacterial activity test of the crude extract of Garlic against both standard and clinical isolates was carried out by the Agar diffusion method (Leven *et al.*, 1979).

2.3.1 Cork borer method

In this method, 0.2 ml of garlic extract was mixed with 20 ml of sterile nutrient agar using a mixer (vortex) and then poured into sterile Petri dishes. After solidifying, the seeded agar was punched out with a sterile cork borer or back hole of 10 ml pipette diameter=9 mm at equally spaced out positions to make four agar wells. Four of the wells were filled with 0.1 ml of the test sample solution while the fifth was with standard antibiotics (chloramphenicol+distilled water) per well. The plates were then left at room temperature for 2hr (to favor diffusion over microbial growth) and incubated in an incubator at 37°C for 24 hr. As mentioned, each sample was done in triplicate, and the antibacterial activity was evaluated by measuring the diameter of the zone of inhibition using a ruler.

3.32 Determination of minimum inhibitory concentrations (mics)

The MIC was determined using the agar well diffusion method. Concentrated ethanolic extracts (100ul) of turmeric were added at two-fold serial dilution (0.244 to 1000 ppt) in approximately 2 mm wide agar wells on assay plates, and incubated at 37°C for 24 h. MICs values were taken as the lowest concentration of extract which completely inhibited bacterial growth after 24 h of incubation at 37 °C.

3. Results

The results show the prevalence of *S. aureus* isolated from urine samples of a female student of Alex-Ekwueme Federal University

Table 1. Prevalence of *S. aureus* isolated from urine samples of female student

No. of Samples examined	No. positive for <i>S. aureus</i>	No. negative for <i>S. aureus</i>
120	90 (75%)	30 (25%)

Table 2a. Antibacterial activity of Garlic and Ginger methanoic extract by agar diffusion method.

isolate source	Microorganism	Concentration in methanoic extract							
		25mg/ml		50 mg/ml		75 mg/ml		100 mg/ml	
		Gl	Gg	Gl	Gg	Gl	Gg	Gl	Gg
Urine	<i>S. aureus</i>	-	-	+	-	+	-	+	+
Standard	<i>S. aureus</i>	+	+	+	+	+	+	+	+

Table 2b. Antibacterial activity of Garlic and Ginger ethanoic extract by agar diffusion method.

isolate source	Microorganism	Concentration in ethanoic extract							
		25mg/ml		50 mg/ml		75 mg/ml		100 mg/ml	
		Gl	Gg	Gl	Gg	Gl	Gg	Gl	Gg
Urine	<i>S. aureus</i>	-	+	-	+	-	+	+	+
Standard	<i>S. aureus</i>	+	-	+	+	+	+	+	+

Table 3a. Antibacterial activity of Garlic and Ginger extract compared with standard drugs against test organisms by Agar well diffusion

Antibiotic type	Concentration	Size	Organism presence		
			<i>S. aureus</i>	Ethanol	Methanol
Garlic	100mg/ml	Small	+	+	-
	75mg/ml	Small	+	+	-
	50mg/ml	Medium	+	+	-
	25mg/ml	Large	-	+	+
Tetracycline	100mg/ml	Very small	-	Nil	Nil
Penicillin	100mg/ml	medium	-	Nil	Nil

+indicates Inhibition while -indicates growth.

Table 3b. Antibacterial activity of Garlic and Ginger extract compared with standard drugs against test organisms by Agar diffusion.

Antibiotic type	Concentration	Size	Organism presence		
			<i>S. aureus</i>	Ethanol	Methanol
Ginger	100mg/ml	Small	+	+	-
	75mg/ml	Small	+	+	-
	50mg/ml	Medium	+	+	-
	25mg/ml	Large	-	+	+
Tetracycline	100mg/ml	Very small	-	Nil	Nil
Penicillin	100mg/ml	Medium	-	Nil	Nil

+indicates Inhibition while -indicates growth

4. Discussion

In this study, the result of Table 1 shows that out of fifty-four urine samples analyzed, thirty-two were positive for *Staphylococcus aureus*. This work is in agreement with the report of Ayogu *et al.*, (2017) and Ani *et al.*, (2015), who isolated 60% of *Staphylococcus aureus* in the urine samples of women attending antenatal care in Mater Misericordia Hospital Afikpo Ebonyi state. Both urine and standard isolates of *S.aureus* was highly sensitive to concentrations of 100 ml/20 ml of agar media using diffusion and Cork borers method (Abebe, 2003). As a result, the garlic extract was more crucial for the prevention of resistant *S. aureus*, which is a problem because it can now acquire resistance to several commercially available medications, including penicillin (Ephrem and Asefaw, 2016).

In this study, we have observed that, as the concentration of the Garlic and ginger extract increases we have seen efficiency increase, and hence inhibition and growth of test bacteria has been diminished. As can be seen from the data above, larger clear zones are present at greater concentrations, and smaller clear zones are present at lower concentrations.

Results of the antimicrobial activity of the Methanoic extract of garlic on *Staphylococcus aureus* revealed high resistance across all concentrations except at 100mg/ml which is the highest concentration, while in Ethanoic extract of ginger reveals high susceptibility across all concentrations. Comparatively, this study reveals that Antimicrobial activity was higher in Methanoic garlic extract as compared to Methanoic ginger extract while Ethanoic ginger extract was observed to be more active as compared with that of garlic though little resistance was observed across all concentrations of the extracts (Ephrem and Asefaw, 2016).

Conclusion

This study shows that garlic ethanolic extract makes large clear zones than currently available antibiotics used. This study also shows that Garlic could be used as an effective antibacterial agent in Nigeria where *S. aureus* is known to be resistant. It could be made in tablet form or encapsulated in the best concentrations and at an affordable dosage and price so that it can be used as medicine for this uropathogenic organism. There is a need to focus on alternative drugs especially in this era of drug resistance to avoid such emerging diseases that could be easily available and inexpensive.

Recommendations

One can use garlic and ginger as part of the daily diet for better health, especially the fresh ones. This research can be used as a base to identify the actual allicin minimum inhibition concentration.

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Ethical clearance

Ethical approval and informed consent of the students were sought and obtained from a female student and hostel management of Alex-Ekwueme Federal University Ndufu-Alike Ebonyi State

Competing Interests

The authors declare no competing interests.

Author's Contribution

All the contributed authors jointly designed the study methodologies and edited and approved the manuscript.

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