Assessing the Phytochemical Profile and Antimicrobial Efficacy of *Allium sativum* Against Some Bacterial Pathogens

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Abstract: This research was carried out to examine the phytochemical composition and antibacterial efficacy of garlic against some bacteria pathogens. A standardized phytochemical investigation was done qualitatively utilizing benchmark protocols. The garlic extract's ability to combat bacterial growth was evaluated using the agar well diffusion assay. Potency of the *Allium sativum* extracts was investigated by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values via micro dilution methodology against selected bacteria pathogens. Phytochemical analysis of the garlic extracts revealed many bioactive constituents, including saponins, flavonoids, tannins, alkaloids, terpenoids, glycosides, anthraquinolones and steroids; which are found diverse levels of presence across various extracts The antimicrobial efficacy of *Allium sativum* extracts (aqueous and ethanol) was evaluated at potency from 25 to 200 milligram/ml, resulting in diameters of inhibition size 10-22 millimeter (*S. aureus*), 9-21 millimeter (*E. coli*). Garlic extracts MIC against *Staphylococcus aureus* ranged from 25 milligram/milliliter (ethanol) to 50 milligram/milliliter aqueous while *E. coli* had a uniform MIC of 25 milligram/milliliter for both extracts. Both *S. aureus* and *E. coli* exhibited MBC values of 50 milligram/milliliter (aqueous extract) and 25 milligram/milliliter (ethanol extract), indicating ethanol extract's enhanced bactericidal potency. The observed antibacterial effects of garlic extracts are likely due to the synergistic action of some of its bioactive compounds; supporting its potential use as a natural antimicrobial agent. The ethanol extract of garlic exhibited superior antimicrobial activity (11-22 mm) compared to the aqueous extract (9-21 mm); displaying concentration-dependent inhibition. Therefore, it is advisable to conduct a thorough structural elucidation of garlic bioactive constituents to assess the actual potency among various phytochemicals discovered in it.

Key word: Ethanol extracts, aqueous extracts, *Staphylococcus aureus, Escherichia coli, Allium sativum*

INTRODUCTION

Medicinal plants are gaining significant attention. This is because they offer a promising alternative to synthetic medications, boasting affordability, minimal side effects, and greater patient acceptability with a high therapeutic potential (Sanusi *et al*., 2017). The growing interest in Ayurvedic medicine, which utilizes around 420 plant species – including garlic – is a prime example of the growing interest in natural health solutions for man's own health benefits (Islam *et al*., 2014).

Phytochemicals present in herbaceous plants comprises tannins, flavonoids, terpenoids, alkaloids, saponins and steroids etc. (Shuaibu, *et al.*, 2019). Medicinal plants harbor bioactive compounds with unique phytochemical properties which varies among different plant species. This unique property exhibited by medicinal plant is as a result of their biodiversity and capacity to induce significant physiological effects in humans (Shuaibu *et al*., 2019).

Across Nigerian cultures and dialects, garlic is referred to as 'Ata ile' in Yoruba, 'Galiki' in Igbo, and 'Tafarnuwa' in Hausa (Fadiji, 2019). It boasts of ancient medicinal uses with broad applications to include antibacterial properties and effectiveness in managing arthrosclerosis, cancer and diabetes (Toledano *et al*., 2019).

Research has shown garlic to have cardiovascular benefits, lowering pressure and blood plasma cholesterol, while also preventing platelet aggregation (Toledano *et al*., 2019). Its bioactive constituents, alliin and alliinase, drive these benefits, supporting traditional uses for garlic in treating various ailments like dental issues and wounds, viral infections, as well as spiritual ailments (Kim *et al*., 2017).

Escherichia coli is a rod-shaped gram negative bacterium that is found in the gut of warm blooded animals. *Staphylococcus aureus* is a non-motile, facultative anaerobes, gram-positive and circular shaped bacterium which is a topical commensal microorganism of the human body, frequently isolated from the skin and nasopharynx (Unegbu *et al.*, 2020).

Antibiotic resistance poses a significant challenge, prompting researchers worldwide to exploring innovative solutions, including the screening of medicinal plants (Unegbu *et al.*, 2019). This quest seeks to uncover novel, effective, and safer therapeutic agents capable of combating infectious diseases (Unegbu *et al.*, 2019). Therefore, the aim of this research was to assess the phytochemical profile and antimicrobial efficacy of *Allium sativum* extracts against some bacteria pathogens.

MATERIALS AND METHODS

Preparation of plant samples: Wholesome garlic bulbs, bought from the open market and certified by a plant biologist were processed in the laboratory by washing, slicing, air-drying, and pulverization into powder using sterile equipment, and stored in a transparent glass bottle for subsequent use (Unegbu *et al.*, 2020).

Collection and confirmation of the model bacteria: Model cultures of *E. coli* and *S. aureus* were sourced at Nnamdi Azikiwe University's Department of Microbiology, Anambra State, Nigeria. Their identities were confirmed through standard microbiological techniques and subsequently preserved at 4°C.on slants containing nutrient agar (CLSI, 2014).

Biochemical characterization of the model bacteria E. coli: For isolation and confirmation, the culture of *E coli* was plated on Eosine Methylene Blue agar and incubated for a period of 24 hrs. Colonies with a distinct green metallic sheen indicated a positive result; and confirmed by sub-culturing onto Chromagar *E. coli* medium (Oxoid, Basingstoke, UK), where they exhibited a blue/violet appearance (CLSI guidelines (2014).

Staphylococcus aureus: Following a positive catalase test result, *S. aureus* was grown on blood agar and incubated overnight 37°C. The isolated colonies were then inoculated onto Mannitol salt agar (MSA) for 24 hours; and a smooth, yellow, circular colonies appearance on MSA showed a positive result, according to CLSI protocols (2014).

Standardization of the model organism: A 24hours old growth broth of *E. coli and S. aureus* were standardized by inoculating the model bacteria into 5 ml of sterile nutrient rich broth and incubating for 2 hours. The turbidity of the broth was calibrated to align to 0.5 McFarland standard, indicating optimal growth (Garba *et al*., 2013).

Synthesis of aqueous and ethanol garlic extracts: Preparation of garlic extracts was done following established protocols as described by Garba, *et al.* (2013).

Preparation of aqueous extract: Garlic powder (10g) was infused in 100ml of distilled water and allowed to steep overnight. The solution was filtered using Whatman filter paper, and the resulting filtrate immediately vaporized by the use of a water bath. The residue was stored in universal sterile container at 4°C until there is need for it (CLSI, 2014).

Preparation of ethanol extract: Garlic powder (10 g) was soaked overnight in 100ml ethanol (95%) and resulting solution filtered to remove solids. The resulting solvent was evaporated; and yielded the garlic extract, which was stored at 4°C (CLSI, 2014).

Extract dilution: The already prepared extracts of garlic (aqueous and ethanol) were diluted with distilled water to a desired concentrations (200 to 25 mg/ml), as established by CLSI (2014).

Sterility test of garlic extract: Both garlic extracts was tested (sterility and contamination) following the protocol outlined by Unegbu *et al.* (2019). A 1-ml sample of each extract was transferred to

N.A plates aseptically and incubated overnight at 37°C. The lack of growth on the plates verified the extracts' sterility.

Qualitative phytochemical analysis of the extracts: A phytochemical screening was conducted to identify various bioactive constituents like tannins, flavonoids, saponins, glycosides, carbohydrates, alkaloids, cardiac glycosides, phenols, phlobatannins, steroids anthraquinones and terpenoids, using the method as outlined by Valentine *et al.,.* (2020).

Antimicrobial efficacy of extract of Allium sativum bulbs: The antimicrobial activity of garlic was analyzed using the in vitro diffusion technique, according to the method of Shuaibu *et al.* (2019). Standardized bacterial cultures (5 millimeters) were evenly introduced and spread into inoculated nutrient agar plates. Six wells (5 mm diameter) were created, and 0.5 ml of garlic extract (250, 100, 50, and 25 mg/ml) were dispensed into each well. Gentamicin (20 mg/ml) was used to indicate positive control while distilled water was used to indicate negative control. The perforated plates were incubated overnight at 37°C and the resulting zones of inhibitions were measured with the help of a meter rule.

Evaluation of minimum inhibitory concentration of the extracts: The MIC of *Allium sativum* extracts against the test organisms was evaluated using the broth dilution assay. Nutrient broth (5 millimeters) was mixed with 5 millimeters of each extract, serially diluted to achieve concentrations of 200-25 mg/ml, and inoculated with a loopful of the test organisms. After 24-hour incubation at 37°C, the lowest concentration inhibiting growth was recorded as the MIC (Shuaibu *et al.,* 2019).

Evaluation of minimum bactericidal concentration (MBC) of extracts: Through sub cultivation of 1 ml of bacterial culture from MIC tubes with no visible growth NA plates, and subsequent overnight incubation (37°C), the MBC was determined. The MBC was identified as the lowest concentration of the sample that inhibited growth upon sub-culturing (Shuaibu *et al.,* 2019).

Statistical analysis: Through the use of one way ANOVA, data were analyzed; and a p-value of ≤ 0.05 was considered significant with the use of SPSS software version 21.0.

RESULTS

Table 1 showed the plant based secondary metabolite screening of the aqueous and ethanol samples of *Allium sativum* bulbs. It showed the existence of different phytochemical constituents like tannin, saponin, terpenoid, alkaloids, flavonoid, steroid and glycoside at different concentrations.

Table 2 illustrates the antibacterial efficacy of *Allium sativum* aqueous and ethanol sample against the two model bacteria. *Allium sativum* aqueous extract exhibited zone of inhibition diameters of 21, 19, 14, and 10 mm against *S. aureus* at concentrations (200, 100, 50, and 25 mg/ml0 respectively. The ethanol extract of *Allium sativum* demonstrated zone of inhibition diameters of 22, 20, 16, and 11 mm against *S. aureus* at concentrations of (200, 100, 50, and 25 mg/ml) respectively. The antibacterial efficacy of aqueous and ethanol samples to *E. coli* is also presented in Table 2.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of garlic bulb samples on the model bacteria are summarized in table 3. For *S. aureus,* MIC & MBC of the aqueous extract were both 50 mg/ml. In contrast, MIC & MBC of aqueous sample against *E. coli* were 25 mg/ml and 50 mg/ml, respectively. MIC & MBC figures of ethanol extract on *S. aureus* and *E. coli* are also summarized in Table 3.

Table 1: Photoactive compounds of ethanol extracts (EE) and aqueous (AE) extracts garlic

Photoactive compounds	AE	EE
Carbohydrate	+	-
Terpenoid	+	++
Tannin	+++	++
Flavonoids	++	+++
Saponins	++	+
Alkaloids	++	+++
Glycosides	+	++
Steroids	+	+++
Anthraquinone	+	+
Phlobatannis	-	-
Phenol	-	-

Key: +++ = high concentration; ++ = moderate concentration; + = trace concentration; - = zero concentration

Table 2: Antibacterial efficacy of garlic bulb extracts against the two model bacteria

	diameter zone of inhibition (millimeter) of extract concentrations						
Isolate	200	100	50	25	+C	-C	Extracts
S. aureus	21± 0.02	19± 0.01	14± 0.05	10± 0.03	17±0.01	0	AE
S. aureus	22± 0.04	20± 0.01	16± 0.02	11± 0.04	17± 0.01	0	EE
E. coli	18± 0.05	15± 0.03	11± 0.04	9± 0.01	18± 0.01	0	AE
E. coli	21± 0.02	17± 0.01	14± 0.03	11± 0.04	18± 0.01	0	EE
P level (0.05)	***	***	***	***	***	***	

Key: +C: reference standard (Gentamicin, 20 mg/ml); -C: baseline control (Sterile water); *** = significant difference at $p \leq 0.05$

Table 3: MIC and MBC values of aqueous and ethanol samples of garlic against the model bacteria

	Extracts concentration in milligram/milliliter		
Isolates	MIC	MBC	Extracts
S. aureus	50 ± 0.02	50± 0.01	AE
S. aureus	25 ± 0.02	25± 0.01	EE
E. coli	25 ± 0.04	50 ± 0.02	AE
E. coli	25 ± 0.04	25± 0.03	EE
p level (0.05)	***	***	

Key: *** = significant difference at $p \leq 0.05$

DISCUSSION

The findings from the phytochemical constituents of the extracts confirmed the existence of rich bioactive agents like steroids, tannins, saponins, alkaloids, flavonoids, terpenoids and glycosides in various concentrations of garlic extracts. This was similar to studies conducted in different part of Nigeria (Akintobi *et al.*, 2013; Fadiji, 2019). The observed antimicrobial efficacy of garlic extracts can be attributed to the presence of phytochemicals such as alkaloids, flavonoids, saponins and steroids, which have been previously reported to exhibit antimicrobial properties (Akintobi *et al.*, 2013).

Alkaloids, such as quinine, ephedrine, morphine, strychnine, and nicotine, possess established therapeutic benefits, including anesthetic, cardio-protective, and anti-inflammatory effects, and are commonly used in clinical settings (Hyunjoo, 2018).

According to Hyunjoo (2018), flavonoids exhibit a range of biological properties, including cytotoxic, antimicrobial, anti-inflammatory and antitumor activities, but their most notable characteristic is their potent antioxidant capacity. Saponins exhibit a broad spectrum of biological activities, including antiprotozoal, antioxidant, antimollusk and antinutrient properties, as well as inducing hypoglycemia, and demonstrating antifungal and antiviral effects (Fadiji, 2019). Similarly, steroids exhibit analgesic and central nervous system activities (Garba *et al.*, 2013).

The bacterial species tested exhibited varying susceptibility to garlic extracts derived from different organic solvents, with ethanol extracts demonstrating significantly higher antimicrobial activity relative to aqueous extracts. Valentine *et al.* (2020) highlighted the constraints of using water as a solvent in seeking innovative antimicrobial agents, citing its ineffectiveness in extracting non-polar compounds.

The antimicrobial efficacy of garlic sample was higher in ethanol extracts than aqueous extracts, inhibiting both model bacteria in a dose-dependent manner. This variation is attributed to differences in phytochemical content between the extracts (Shrestha *et al.*, 2016). This finding was similar to the work of Martins *et al.* (2016).

This research showed that ethanolic extracts of garlic exhibited significantly enhanced zones of inhibition than aqueous extracts at all tested concentrations, illustrating their potential as a novel, natural antimicrobial agent. Comparable results were reported by Szychowski *et al.* (2018). This study revealed varying effective concentrations against both model bacteria used in this research work. Notably, the ethanol extracts exhibited low MIC and MBC values, which suggest potent antibacterial activity, supporting the findings of Elzein *et al.* (2018). The differences in MBC values obtained for garlic extract against *E. coli* and *S. aureus* suggest variations in phytochemical properties, leading to distinct inhibitory and bactericidal effects on the test organisms, outperforming standard antibiotics (Abubakar and Usman, 2016).

The variation in antimicrobial effects among the extracts can be further explained by differences in their chemical composition, as noted by Abiola *et al.* (2017). This corroborates the work of El-Hamidi and El-Shami (2015) and Abiola *et al.* (2017), which showed that garlic extracts exhibit potent bactericidal effects, outperforming those of standard antibiotics.

CONCLUSION

The observed antibacterial effects of garlic extracts are likely due to the synergistic action of secondary metabolites, including tannins, flavonoids, saponins, terpenoids, glycosides, steroids, alkaloids, and anthraquinones; supporting its potential use as a natural antimicrobial agent. Therefore, it is advisable to conduct a thorough structural elucidation of garlic bioactive constituents to reveal the real bactericidal activities of various phytochemicals discovered in it. In addition, pharmaceutical companies should channel more resources to the developments of novel drugs which has garlic as its natural origin.

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