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Antimicrobial and Phytochemical Activities of Garlic (*Allium sativum*) on *Staphylococcus aureus* and *Candida albicans* Isolated from High Vaginal Swab samples and Female Students with UTI

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Abstract **Original Research Article**

Studies on antimicrobial and phytochemical activities of garlic on *Staphylococcus aureus* and *Candida albicans* isolated from HVS samples and female students with urinary tract infection were investigated. Garlic is used in many homes as condiment or as flavoring agent. The garlic used was purchased from Eke market Agbani in Nkanu West Enugu State Nigeria. Ten Urine and HVS samples were collected from female students using a universal container and swab sticks. Preliminary qualitative phytochemical analysis was done using standard methods. The powder of garlic was macerated in ethanol and chloroform to produce crude extract reconstituted with distilled water to concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml. The ethanol and chloroform extract of garlic obtained was used to evaluate antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*. Also, minimum inhibitory concentration was determined. The garlic extract contained alkaloids, tannins, flavonoids, terpenoids and saponins. The inhibitory effect of ethanol extract started at 200mg/ml with inhibition zones of 2mm against *Staphylococcus aureus* and *Candida albicans.* MIC result for garlic extract on *Staphylococcus aureus* and *Candida albicans* showed that the extract has inhibitory effect at low concentration of 50mg/ml and inhibition increased at 100mg/ml. Ethanol extract was the most effective extract retarding microbial growth of *Staphylococcus aureus* and *Candida albicans*. This study however, justify the use of garlic extract in traditional medicine practice as a therapeutic agent. In cases where possible, the ethanol extracts of garlic should be used at a concentration up to 100mg/ml so as to give a better treatment.

Keywords: Garlic, Ethanol, Chloroform Phytochemical, Antimicrobial Activities, *Staphylococcus Aureus* and *Candida Albicans*.

INTRODUCTION

Herbal drugs have found wide spread use in many countries because they are easily, available, cheaper, and safer than synthetics drug (Retnam and De-Britto, 2017; Prusti *et al.,* 2018). According to World Health Organization (WHO) about 80% of people worldwide are currently depending on traditional medicine for their primary health care needs (Khalil *et al.,* 2017). Antimicrobial resistance has been a major concern, a large number of bacteria have responded to the use of antibiotics with their ability to evolve and transmit antibacterial resistance to other species (Nerino *et al.,* 2013).

Allium sativum commonly known as Garlic which belongs to a family of Alliaceae, has more than 500 species in 30 genera (Divya *et al.,* 2017). It is widely used in culinary and medicine; it has been utilized to fight infection such as cough, cold asthma, diarrhea, flu, headache sore throat, abdominal discomfort and respiratory infection (Bandna, 2013). Garlic is probably one of the earliest known medicinal plants, which used for ancient time to cure different disease condition in human. Garlic's principal medicinal uses are to lower blood pressure and cholesterol and prevent cancer (Gavasane *et al.,* 2011).

The most common active components of fresh garlic are an amino acid called alliin and an enzyme like peroxidase, allinase, myrosinase and other compounds like αphallandrene, βphallandrene, linalool, citral and geraniol. When a clove of garlic is chewed, chopped, bruised, or cut, these compounds mix to form allicin,

which is responsible for its strong smell. Within a few hour allicin, breaks down into other sulfur compounds with variety of overlapping healing properties. *Allium sativum* also contains a wide range of trace minerals which include copper, iron, zinc, magnesium, germanium, and selenium. Soil rich with the presence of trace minerals will produce a healthful bulb of *Allium sativum*, full of those minerals. In addition, garlic contains many sulphur compounds, vitamin A and C, and various amino acids (Gavasane *et al.,* 2011).

Bulbs of garlic are reported to contain terpenoid in extracts. Terpenoids have been found to be useful in therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties. Flavonoids are also present in extracts as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anti-cancer activity (Okwu, 2001). Tannin and saponin are present in *Allium sativum* extract. Saponins protect against hypercholesterolemia and antibiotics properties. In addition, it has been found that saponins have antitumor, antioxidant and anti-mutagenic activities and can lower the risk of human cancers by inhibiting the growth of cancer cells (Okwu, 2001).

A. sativum had been reported to inhibit the growth of multidrug-resistance bacteria of both Gram-negative and Gram-positive bacteria. The plant species have shown promising micro biostatic and microbiocidal activities against a range of pathogenic microbes and these are attributed to the presence of minute doses of bioactive contents referred to as phytochemicals (Omojate *et al.,* 2014).

MATERIALS AND METHODS

Collection and Transportation of Samples

The garlic (Allium sativum) was purchased from Eke market Agbani in Nkanu West Local Government Area of Enugu State. The samples were transported to Applied Biology laboratory of Enugu State University of Science and Technology immediately after collection for identification. The sample was washed thoroughly, cut into smaller portions using a sterile scalpel blade, and sundried. While Different Urine samples were randomly and carefully collected from ten (10) female ESUT students using a sterilized universal container.

Isolation of the Organisms from the Urine and HVS Samples

This was carried out as described by (Durairaj, *et al.,* 2009). The urine and HVS samples were serial diluted in 10-fold and cultured on Nutrient agar, Potato Dextrose Agar and Mannitol Salt and incubated for 48 hours at 37^{o}C. After incubation, discrete colonies were sub cultured on Mannitol salt agar and Potato Dextrose Agar, to obtain pure culture. The sub-culturing of the isolates was done by using pour plate method. Mannitol salt agar was then incubated at 37^{o}C for 24 hours, while PDA was incubated for 28 ^{o}C for 48 hours.

Identification of the Isolates from the Urine and HVS Samples

The identification was done using a method described by (Cheesbrough, 2009). The isolates were identified and characterized based on the morphological and biochemical test which also include Lacto phenol cotton blue, Gram staining, Indole test, Catalase, test and Oxidase test, Urease test, Citrate Utilization test, Methyl red test, Glucose fermentation test.

Ethanol and Chloroform Extraction of Allium Sativum

Extraction was carried out according to method described by Melvin *et al.,* (2009). 100gm of Garlic bulbs were coarsely pondered using mortar and pestle, coarsely sample were soaked with 500ml ethanol and Chloroform solution respectively, stirred and allowed to stand for 24 hours. The suspension was filtered using filter paper. The filtrate was evaporated under reduced pressure. The concentrated extract was stored in a labeled sterile screw capped bottle at 2- 8°C.

Phytochemical Analysis of the Extract

The determinations were done by utilizing standard methods illustrated by (Preshant *et al.,* 2011). The preliminary analysis involved testing for the presence of flavonoids, terpenoids, steroids, saponins, alkaloids, tannins, glycosides and phenols.

Test for Tannins

Extract (0.1 g) was stirred with 10 ml of distilled water and then filtered. Few drops of 1 % ferric chloride solution were added to 2 ml of each filtrate. The presence of a blue-black or blue-green precipitate indicated the presence of tannins (Avato, *et al.,* 2006).

Test for the Alkaloids

A quantity of the extract (0.1 g) was dissolved individually in dilute hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids (Preshant *et al.,* 2011).
b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids (Preshant *et al.,* 2011).
c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Avato, *et al.,* 2006).

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate (Avato, *et al.,* 2006).

Test for Saponins

A quantity of each extract (0.1 g) was boiled with 5 ml of distilled water and filtered. To each filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins (Avato, *et al.,* 2006).

Test for Glycosides

Each extract (0.1 g) was mixed with 30 ml of distilled water and heated on a water bath for 5 minutes. To 5ml of each of the filtrates, 0.2 ml of Fehling's solution A and B was added until it turns alkaline. The solutions were heated on a water bath for 2 minutes. A brick – red precipitate indicated the presence of glycoside (Preshant *et al.,* 2011).

Test for Terpenoids

Each extract (0.1 g) was dissolved in ethanol. Acetic anhydride (1 ml) was added, followed by the addition of concentrated H2SO4. A change in colour from pink to violet showed the presence of terpenoids (Preshant *et al.,* 2011).

Lead Ethanoate Test for Flavonoids

A quantity (0.1 g) of each extract was dissolved in water and filtered. To 5 ml of each of the filtrates, 3 ml of lead ethanoate solution was added. Appearance of a buff – coloured (pale yellow-brown) precipitate indicated the presence of flavonoids (Avato, *et al.,* 2006).

Liebermann-Buchard Test for Steroids

0.1 g of each extract was added to 2 ml of acetic acid. The solution was cooled well in ice followed by the addition of concentrated tetraoxosulphate (VI) acid (H2SO4) carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring (Avato, *et al.,* 2006).

Ferric Chloride Test for Phenols

About 0.1 g of each extract was boiled with distilled water and then filtered. To 2 ml of each filtrate, few drops of 10 % ferric chloride solution were then added. A green – blue or violet colouration indicated the presence of a phenolic hydroxyl group (Avato, *et al.,* 2006).

Preparation of Different Concentration for the Extract

200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml and 12.5mg/ml concentrations of the crude extract of ethanol and chloroform was prepared by dissolving 6g of the extract in 10ml of 5% DMSO respectively.

Preparation of Antimicrobial Sensitivity Test

Antimicrobial was performed using agar well diffusion method according to (Agalu *et al.,* 2014) for ethanol extract and chloroform extract. The 0.5 Macfarland turbidity standards were used to adjust the turbidity of the inoculm for the antimicrobial susceptibility test. The 0.5 MacFarland turbidity standard was prepared by adding 0.5ml of a 1.1758 (wt/vol) Barium chloride dehydrate (BaCL2 2H2O) solution to 99.5ml of 1% (vol/vol) sulphuric acid (H2SO4). The turbidity standard was then aliquoted into screw, capped test tubes identical to those used to prepare the inoculum suspensions; the test tubes were then sealed with wax to avoid evaporation. Inoculating needle was used to select the isolated colonies and these were transferred into test tubes containing nutrient broth. They were vortexed thoroughly. The test tube containing the turbidity standard was also vortexed so that the white precipitate of barium sulphate could be mixed well. The organisms' suspensions were then compared to 0.5 McFarland standards for turbidity. During comparison, those bacterial suspensions that did not appear to be the same density as the 0.5 MacFarland standards were either reduced by adding sterile saline or increased by adding more bacterial cells. Within 15 minutes after adjusting the turbidity of the inoculum suspension, sterile cotton swabs were dipped into the various test tubes containing the organisms. The sterile cotton swab was pressed firmly against the inside wall of the test tubes just above the fluid level. The swab: were rotated to remove excess fluid. Each of the swabs was then streaked over the entire surface of the various agar plates (Muller-Hinton agar). The plates were rotated at 60°C after each application to ensure even distribution of the inoculum. After the inoculation, a cork borer was used to bore six holes in which the stock (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of the extract dilution antibiotics (flouconalzole for fungi, and gentamicin for bacteria) and distilled water was dispensed to the six holes using a syringe and it was incubated at 37°C for 24 hours. Clear zones of inhibition produced by the organisms were observed and measured.

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of the ethanol and chloroform extracts garlic was determined using tube dilution method as described by (Agalu *et al.,* 2014). Decreasing concentrations of the extracts used in the antimicrobial assay that exhibited inhibition (100mg/ml) was prepared using two-fold dilution method using Mueller Hinton broth. Six sterile test tubes were used with each containing 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml concentrations of the ethanol and chloroform extract respectively. Two tubes served as control: nutrient broth inoculated with bacteria was used as a positive control and nutrient broth containing the extracts was used as a negative control. After incubating for 18 hours at 37°C, the tubes were examined for turbidity indicating the growth of the

microorganisms. The lowest solution of the extract that inhibited the growth of the microorganism as detected by the lack of visual turbidity (matching the negative growth control) was designated the minimum inhibitory concentration.

Determination of Minimum Bactericidal Concentration (MBC)

According to (Agalu *et al.*, 2014). The minimum bactericidal concentration was determined by assaying the test tubes resulting from MIC determination. A loopful of the content of each test tube was inoculated by streaking on a solidified Mueller Hinton agar plate and then incubated at 37 °C for 24 hours

RESULTS

The morphological characteristics of the *Staphylococcus aureus* and *Candida albicans* are shown in Table 1.

Table 1: Morphological Characteristics of the *Staphylococcus aureus* and *Candida albicans*

Isolates	Macroscopic Characteristics	Microscopic Characteristics
Staphylococcus aureus	Consisted of smooth, golden yellow colonies on nutrient agar and also appeared yellow on manitol salt agar medium.	Consisted of Gram positive cocci in clusters which appeared purple.
Candida albicans	Consisted of thick, white appearance on potato dextrose agar medium.	Consisted of round to oval large budding yeast which appeared purple.

The biochemical characteristics of *Staphylococcus aureus* and *Candida albicans* are shown in Table 2

Table 2: Biochemical Characteristics of *Staphylococcus aureus* and *Candida albicans*

Isolates	Gram Stain	Indole test	Catalase test	Oxidase test	Urease test	Citrate Utilization test	Lactophenol Cotton Blue Stain	Coagulase test	Haemolysis test
Staphylococcus aureus	+	-	+	-	+	+	**NT**	+	+
Candida albicans	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	+	**NT**	**NT**

KEYS (-)Negative,
(+)Positive
(NT) Not tested

The phytochemical analysis of ethanol and chloroform extracts of garlic was shown in Table 3.

Table 3: Phytochemical analysis of the Extracts

Phytochemica	Garlic Ethanol extract	Garlic Chloroform Extract
Alkaloids	+++	++
Phenols	+	-
Glycosides	+++	+
Tannins	++	+
Saponins	++	++
Terpenoids	+	++
Steroids	++	+++
Flavonoids	+++	++

KEYS
-:Absent
+:Slightly present
++:Moderate
+++:Abundant

Antimicrobial activity of ethanol and chloroform extracts of garlic on *Staphylococcus aureus* are shown in Table 4.

Table 4: Antimicrobial Activity of Garlic Extracts on the Isolates

Isolates				**Concentrations**		
Staphylococcus aureus	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	Standard Drug	Control
Ethanol	5mm	3mm	2mm	0mm	26mm	0
Chloroform	6mm	4mm	2mm	0mm	18mm	0

Standard drug: Gentamicin
Control: Distilled: Water

Antimicrobial activity of ethanol and chloroform extracts of garlic on *Candida albicans* are shown in Table 5.

Table 5: Antimicrobial Activity of Garlic Extracts on the Isolates

Isolates				**Concentrations**		
Candida albicans	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	Standard Drug	Control
Ethanol	5mm	3mm	2mm	1mm	21mm	0
Chloroform	7mm	5mm	3mm	1mm	16mm	0

Standard drug: Flocunazole
Control: Distilled water

The minimum inhibitory concentration and minimum bactericidal concentration of the garlic extracts on *Staphylococcus aureus* and *Candida albicans* are shown in Table 6

Table 6: MIC and MBC of the Garlic Extracts on *Staphylococcus aureus* and *Candida albicans*

Isolates	**Different concentrations of the Garlic Extracts tested against *Staphylococcus aureus* and *Candida albicans* with reference to MIC and MBC**					**MIC**	**MBC**
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml		
Staphylococcus aureus	-	+	+	-	-	50mg/ml	100mg/ml
Candida albicans	-	-	+	+	-	25mg/ml	50mg/ml

KEYS: + Inhibiton
– No inhibition

DISCUSSION

Studies were conducted on antimicrobial and phytochemical activities of garlic (Allium sativum) against *Staphylococcus aureus* and *Candida albicans* isolated from high vaginal swab samples and female students of Enugu State University of Science and Technology with urinary tract infection. The isolates were morphologically and biochemically identified as *Staphylococcus aureus* and *Candida albicans* using standard identification techniques (Tables 1-2). These relates with the work of Cheesbrough, (2000).

The phytochemical analysis of ethanol and chloroform extracts of garlic were conducted and result showed that Alkaloids, Glycosides and Flavonoids were abundant; Saponin Tannin and Steroids were found in moderate amounts, while Terpenoids were slightly present and Phenols were absent (Table 3). These are in line with the work of (Mohammed et al., 2016).

Antimicrobial activity of ethanol and chloroform extracts of garlic on *Staphylococcus aureus* was carried out using the agar well diffusion method with the ethanolic and chloroform extracts at concentrations of 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml result showed inhibition zones of (ethanol) 5 mm, 3 mm, 2 mm, and 0 mm (chloroform) 6 mm, 4 mm, 2mm and 0 mm against *Staphylococcus aureus* respectively while ethanolic and chloroform extract at 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml exhibiting inhibition zones of (ethanol) 5mm, 3mm, 2mm, and 1mm (chloroform) 7mm, 5mm , 3mm and 1mm against *Candida albicans* (Tables 4-5). These relates with the work of (Canizares et al., 2012).

Studies were also carried out to determine the minimum inhibitory concentration and minimum bactericidal concentration of garlic extracts on *Staphylococcus aureus* and *Candida albicans*, results showed that the minimum inhibitory concentrations of *Staphylococcus aureus* and *Candida albicans* were observed at concentration 50mg/ml and 25mg/ml the extract was able to inhibit the growth of *Staphylococcus aureus* and *Candida albicans*. The Minimum bactericidal concentration of *Staphylococcus aureus* and *Candida albicans* were observed at concentration of 100mg/ml and 50mg/ml (Table 6). The findings partly agree with the work of (Lanzotti et al., 2014).

CONCLUSION

This study showed that there was antimicrobial activity of ethanolic and chloroform extract of A. sativum (garlic) against isolates of *Staphylococcus aureus* and *Candida albicans* probably due to the presence of phytochemicals such as Alkaloids, Flavonoids, and Glycoside. It can be concluded from this study that ethanol extracts of Garlic can be used as substitute for the existing conventional drugs already in use for the treatment of disease. Further studies in A. sativum should be done by using different methods and different solvents for extraction process.

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