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Research Paper

Comparative assessment of the antibacterial profile of Onion (*Allium cepa*) and Garlic (*Allium sativum*) against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*

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Abstract. Aqueous and ethanol extracts of onion and garlic were evaluated for antibacterial susceptibility profile using disc dilution method. Each extract was evaluated at concentrations of 100%, 50% 25% and 12.5% against three common bacterial opportunistic pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*). Result showed that *Pseudomonas aeruginosa* and *Staphylococcus aureus* strain had 25% sensitivity in onion aqueous extract while *Candida albicans* was resistant to it. *Candida albicans* showed 25% sensitivity in onion ethanol extract while *Pseudomonas aeruginosa* and *Staphylococcus aureus* were resistant to it. Both *Pseudomonas aeruginosa* and *Staphylococcus aureus* demonstrated 75% sensitivity to concentrations of aqueous garlic extract, with *Candida albicans* having 50% sensitivity. Result findings revealed that garlic aqueous extract had the highest antibacterial effect on test organisms, which indicates that it could be used in the treatment of diseases associated with the organisms; *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. This explains the basis for the use of these extracts in traditional medicine.

Keywords: Antimicrobial profile, *Allium cepa*, *Allium sativum*, Opportunistic bacteria and traditional medicine.

Introduction

Medicinal plants are of great importance to the health of individuals and communities in general. The antimicrobial activities of such plant extracts have been linked to the presence of bioactive compounds which sometimes serve to protect the plants themselves against bacteria, fungi and viral infections as well as exhibiting their antimicrobial properties on these organisms (El-Mahmood *et al.,* 2007). *Allium* is the largest and most important representative of the Liliaceae family comprises of 450 species. Onion (*Allium cepa*) is a bulbous plant widely cultivated in almost every country of the world (Hannan *et al*., 2010). It is rich in proteins, carbohydrates, sodium, potassium and phosphorus (Lampe, 1999). It is among the oldest cultivated plants used both as food and for medicinal applications (Lanzotti, 2006). Onion bulbs contain a good number of phytochemicals, most of which are hydrocarbons and their derivatives. These secondary metabolites include; tannins, terpenoids, alkaloids, flavonoids allins, steroid saponins, polysaccharides fructosans, saccharose and other sugars, which have been found in-vitro to have antioxidant, antibacterial and anti-inflammatory properties (Cowan, 2001).

Onion is documented to have anti-pathogenic (Shams-Ghahfarokli *et al*., 2006; Irkin and Arslan, 2010), anti-cancer (Kodali *et al*., 2015), anti-diabetic (Hague *et al*., 2011), anti-depressant (Jaouad, 2010), cardio protective (Park *et al*., 2009) and hepatoprotective (Adaramoye, 2009) activities. Shams-Haidari *et al*., (2008) deposited that serum uric acid levels significantly lowered on administration of onion to artificially induced hyperuricemic rats. Singh *et al.*, (2009) reported that the peels of red onion had excellent antioxidant and antimutagenic ability which was due to its high concentration of polyphenolic compounds.

Garlic (*Allium sativum*) is classified as a member of family Alliaceae (Huzaifa *et al*., 2014). It is a bulbous plant that produces hermaphrodite flowers, and pollinated by bees and other insects. Research reported that it may be originally native to Asia, but has long been naturalized to Europe northern Africa, Mexico and all over the world (Daka, 2011). Garlic has anti-bacterial, anti-viral, anti-fungal, anti-protozoal, anti-cancer, anti-oxidant, immunomodulatory, anti-inflammatory, hypoglycemic and hypocholesterolemic effect (Rehman and Munir, 2015; Shaloo *et al*., 2015). The herbal medicine may be in the form of powders, liquid or mixtures which may be raw or boiled, ointments linings and incision (Jehan *et al*., 2011).

Phytochemical screening of Garlic by different researchers have revealed the presence of saponin, flavonoids, alkaloids, terpenoid, tannins, phlobutanin, ketone, amino acid and protein, volatile oil and cardiac glycosides (Arify *et al*., 2018; Igra and Zikhi, 2019). Flavonoids have antioxidant activities as well as much health promoting effects viz., anti-allergic, anti-cancer, anti-oxidant, anti-inflammatory, anti-thrombotic, vasoprotective, tumour inhibitory, antimicrobial and antiviral properties (Trease and Evans, 2002). They act on bacteria by inhibiting its protein synthesis (Hong-xi and Song, 2001). The presence of alkaloids in Garlic aqueous bulb extract shows the potential of the extract to have analgesic, antiinflammatory and adaptogenic effects, which helps the host (man and animal) to develop resistance against diseases and endurance against stress (Gupta, 1994).

MATERIALS AND METHODS

Collection of samples

Allium cepa (onion) and *Allium sativum* (garlic) were obtained from Watt market, Calabar, Nigeria, and they were of analytical standard.

Test organisms

Multidrug resistance bacteria; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* were isolated from patients' wounds at the University of Calabar Teaching Hospital (UCTH) Calabar, Nigeria.

Extraction of *Allium cepa* and *Allium sativum*

The onion bulbs were washed with sterile distilled water repeatedly. The outer covering of the bulb was manually peeled off and fleshy part was re-washed with sterile distilled water. The onion bulb was sliced and blended. A 130.4g (65.2g for aqueous and 65.2g for ethanolic extraction) of paste was pressed using muslin cloth. The fresh onion juice was immediately analyzed for its antibacterial activity.

Fresh garlic were thoroughly washed with sterile distilled water. They were cut into pieces to allow easy blending. Exactly 56.8g (28.4g for aqueous and 28.4g for ethanolic extraction) of the blended garlic was pressed using muslin cloth to form juice. The fresh garlic juice was immediately analyzed for its antibacterial activity.

Preparation of Whatman filter paper discs

This was done by placing 2ml of the stack extract (100%) in the first tube, while each of the other three tubes was served with 1ml of distilled water each. Later, 1ml was transferred from tube1 to tube 2, 1ml from tube 2 to tube 3 and finally, 1ml from tube 3 to tube 4, giving concentrations of 100%, 50%, 25% and 12.5% respectively. This method was used for each of the extracts (i.e; onion aqueous, onion ethanolic, garlic aqueous and garlic ethanolic). Each transfer was followed by thorough and gentle shaking to allow for homogenous mixing of contents. Next, filter paper discs already prepared were suspended (soaked) in each of the tubes and allowed to stay for 24 hours, to enable the discs absorb the ingredients of the extracts. Thereafter, the discs were brought out on trays and dried in the oven at 60^{o}C for 10 minutes. After drying, they were brought out of the oven, into their respective sterile containers in readiness for antibacterial sensitivity testing (Kirby-Bauer *et al.*, 1996).

Antibacterial susceptibility testing of *Allium cepa* and *Allium sativum* against selected bacterial isolates

The antibacterial susceptibility profile was determined using disc diffusion method (Kirby-Bauer *et al*., 1996). In this method, sterilized Petri-dishes were set out on the bench and the plates labeled accordingly with their respective bacterial isolate. Then, 1ml of each broth culture of the three isolates was placed on separate plates using three sterile pipettes. Next, Mueller Hinton agar (already sterilized) and held at a temperature of 45 – 55^{o}C was poured into each of the plates and the plates shaken back and forth (swirling) for the contents of the Petri-dishes to mix thoroughly. The plates were then allowed to stand for 30-45 minutes in order to solidify. After solidification, filter paper discs carrying various

Table 1. Antibacterial susceptibility profile of aqueous extract of Onion.

Isolates	100% (mm)	50% (mm)	25% (mm)	12.5% (mm)	Control (µg/ml) (mm)	% Sensitivity
Pseudomonas aeruginosa	6(R)	23(S)	6(R)	6(R)	30(S)	25
Staphylococcus aureus	21(S)	6(R)	6(R)	6(R)	25(S)	25
Candida albicans	6(R)	6(R)	6(R)	6(R)	35(S)	0
% Susceptibility	33.3	33.3	0	0		

Ciprofloxacin (CPX) 10µg/ml, broad-spectrum for both Gram positive and Gram negative bacteria.
R ≤ 7mm; S ≥ 8mm

Table 2. Antibacterial susceptibility profile of ethanol extract of Onion

Isolates	100% (mm)	50% (mm)	25% (mm)	12.5% (mm)	Control (µg/ml) (mm)	% Sensitivity
Pseudomonas aeruginosa	6(R)	6(R)	6(R)	6(R)	31(S)	0
Staphylococcus aureus	6(R)	6(R)	6(R)	6(R)	25(S)	0
Candida albicans	6(R)	6(R)	6(R)	15(S)	23(S)	25
% Susceptibility	0	0	0	33.3		

Ciprofloxacin (CPX) 10µg/ml, broad-spectrum for both Gram positive and Gram negative bacteria.
R ≤ 7mm; S ≥ 8mm

Table 3. Antibacterial susceptibility profile of aqueous extract of Garlic

Isolates	100% (mm)	50% (mm)	25% (mm)	12.5% (mm)	Control (µg/ml) (mm)	% Sensitivity
Pseudomonas aeruginosa	25(S)	23(S)	6(R)	25(S)	30(S)	75
Staphylococcus aureus	30(S)	35(S)	25(S)	6(R)	33(S)	75
Candida albicans	27(S)	28(S)	6(R)	6(R)	29(S)	50
% Susceptibility	100	100	33.3	33.3		

Ciprofloxacin (CPX) 10µg/ml, broad-spectrum for both Gram positive and Gram negative bacteria.
R ≤ 7mm; S ≥ 8mm

concentrations of the extracts such as 100, 50, 25 and 12.5% including the control (Ciprofloxacin) were transferred to appropriate locations on the agar plates with the help of sterile forceps. The plates were then incubated at 37ºC for 24 hours. At the end of incubation, zones of inhibition (for sensitive isolates) or no inhibition (for resistant isolates) were measured and recorded in millimeters.

Determination of Minimum Inhibitory Concentration (MIC) of extracts

The MIC of the fresh onion and garlic juice against the test bacteria was determined using the broth dilution method. One (1.0) ml of the juice was added to 1 ml of nutrient broth and subsequently transferred. One (1.0) ml from the first test tube to the next, for up to the seventh test tube. Then 1 ml of standardized 18hrs broth culture of test organism (1.0 x 106CFU/ml) was inoculated into each test tube and thoroughly mixed on a vortex mixer. The test tubes were then incubated at 37ºC for 24 hrs. The tube with of the lowest dilution with no detectable growth was considered as the Minimum Inhibitory Concentration.

RESULTS

The result of the antibacterial sensitivity profile of aqueous extract of onion (Table 1) showed that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were 25% sensitive to aqueous extract of onion while *Candida albicans* was resistance to all the concentrations of the extract. All text organisms were sensitive to the control. The test organisms demonstrated resistance to most concentrations of ethanolic extract of onion except *Candida albicans* which was 25% sensitive as shown in Table 2.

The aqueous extract of garlic demonstrated high efficacy against test organisms (Table 3), with *Pseudo-*

Table 4. Antibacterial susceptibility profile of ethanol extract of Garlic

Isolates	100% (mm)	50% mm)	25% (mm)	12.5% (mm)	Control (µg/ml) (mm)	% Sensitivity
Pseudomonas aeruginosa	6(R)	6(R)	6(R)	6(R)	30(S)	0
Staphylococcus aureus	6R)	6(R)	6(R)	6(R)	28(S)	0
Candida albicans	6(R)	13(S	6(R)	6(R)	23(S)	25
% Sensitivity	0	33.3	0	0		

Ciprofloxacin (CPX) 10µg/ml, broad-spectrum for both Gram positive and Gram negative bacteria.
R ≤ 7mm; S ≥ 8mm

Table 5. Minimum Inhibitory Concentration (MIC) of onion aqueous extract

Isolates	MIC(µg/ml)	MBC(µg/ml)
Pseudomonas aeruginosa	25.0	50.0
Staphylococcus aureus	6.25	12.5
Candida albicans	50.0	100.0

Table 6. Minimum Inhibitory Concentration (MIC) of onion ethanolic extract

Isolates	MIC(µg/ml)	MBC(µg/ml)
Pseudomonas aeruginosa	25.0	50.0
Staphylococcus aureus	50.0	100.0
Candida albicans	6.25	12.5

Table 7. Minimum Inhibitory Concentration (MIC) of Garlic aqueous extract

Isolates	MIC(µg/ml)	MBC(µg/ml)
Pseudomonas aeruginosa	1.17	3.13
Staphylococcus aureus	3.13	6.25
Candida albicans	1.57	3.13

monas aeruginosa and *Staphylococcus aureus* recording 75% sensitivity to different concentrations of the extract while *Candida albicans* showed 50% sensitivity to the extract. All the test organisms were 100% sensitive to 100% and 50% concentrations of the extract. Result showed that all the test organisms except *Candida albicans* (25%) were resistant to the different concentrations of garlic extract (Table 4).

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) analysis of the aqueous extract of onion (Table 5) revealed that the MIC of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* was 25.0, 6.25, 50.0ug/ml respectively. The MBC analysis revealed that *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* are 50.0, 12.5 and 100.0ug/ml respectively. The MIC and MBC value of ethanolic extract of onions are shown on Table 6. Those of aqueous and ethanolic extracts of garlic are shown on Tables 7 and 8 respectively.

DISCUSSION

This research study revealed that the aqueous extract of garlic had the best antibacterial effect on test organisms; *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* when compared to the ethanolic extract and the values obtained from both aqueous and ethanol extracts of onion. This could be due to the phytochemical

Table 8. Minimum Inhibitory Concentration (MIC) of Garlic ethanolic extract

Isolates	**MIC(**µg/ml)	**MBC(**µg/ml)
Pseudomonas aeruginosa	50.0	100.0
Staphylococcus aureus	50.0	100.0
Candida albicans	25	50.0

constituents of these plants extracts and their different reactivity, solubility and volatility with organic and aqueous medium. This result corroborates the findings of Oboh and Abula, (1997), which attributed the effectiveness of water (aqueous) extract in inhibiting bacteria to the fact that water is a polar solvent and the phytochemical constituents of garlic such as Sulphur, allinase, persoxidase, myrosinase, allicin, ciral, geraniol, linalool, A-phellandrene and B-phellandere (major components) are very soluble in it, hence retaining most of the antimicrobial (antifungal) properties during the extraction process. In a related study, the antimicrobial properties was attributed to the fact that allicin, the main phytochemical constituent of garlic is volatile and very soluble in ethanol and water, thereby maintains its antimicrobial property in inhibiting some gram positive bacteria and some fungi species (Onyeagba *et al*., 2004). The significant effect of garlic on *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albican*s compared with onion is in agreement with the findings of Wolde *et al*. (2018), which reported that Garlic could be used as an effective antibacterial agent for human pathogenic bacteria. It also corroborates the position of Khan *et al.*, (2007) who observed that garlic is effective against some Gram positive and Gram negative bacteria and some fungi such as *Aspergillus niger* and *Aspergillus flavus*. The reduced effectiveness of onion could be attributed to the solubility and volatility of its phytochemical components such as allins, flavonoids, steroid sapronins, tannins, terpenoids, alkaloids, polysaccharides, fructosans, saccharose and other sugars (major components), some of which tend to be lost during the process of extraction especially in organic solvents as explained by Song *et al*. (2000).

This result is also in line with the findings of Reuter *et al*. (1996), which deposited that Garlic has been found to inhibit *Aerobacter, Aeromonas, Bacillus, Citrella, Citrobacter, Clostridium, Enterobacter, Esherichia, Klebsiella, Lactobacillus, Leuconostoc, Micrococcus, Mycobacteriium, Proteus, Providencia, Pseudomonas, Salmonella, Serratia, Shigella*, *Staphylococcus, Streptococcus* and *Vibrio*. The combination of garlic with antibiotics led to partial or total synergism (Didry *et al*., 1992). The antimicrobial activities have been reported to increase with increase in the concentration of extracts (Bakht *et al*. 2014). Benmalek *et al.* (2013) reported that Gram positive bacteria are more sensitive to onion extract response than gram negative bacteria. In a separate study, Anuja *et al.* (2015) had reported that the fresh juices of white onion (*Allium cepa*) and garlic possess significant antibacterial potency against multidrug resistant bacteria.

Zhou *et al.* (2011) in a meta-analysis of garlic found decreased rates of gastric cancer associated with garlic intake. In a 2013 meta-analysis of epidemiological studies, Zhou *et al*. (2013) also observed that garlic intake was associated with decreased risk of prostate cancer. A 2014 meta-analysis of observational and epidemiological studies found out that garlic consumption was associated with a lower risk of stomach cancer in Korean people (Woo *et al*., 2014). Izzo *et al.* (2004) concluded that consumption of both garlic and onion may have anticarcinogenic effects on health. Nitric oxide scavenging might be the reason for its anti-carcinogenic activity as identified by Lee *et al*. (2009). In a study conducted by Fu (2004), it was reported that hot air dried onion powder can strongly inhibit the leukemia cell growth. He also stated that onion could be used in powder form for medicinal purposes.

The result of the Minimum Inhibitory Concentration (MIC) corroborates the findings of Anuja *et al.* (2015), which deposited that the Minimum Inhibitory Concentration (MIC) of garlic was low, ranging from 1.125 – 25 %v/v and that of white onion was 2.125-50%v/v. In a related study, Adesina *et al.* (2011) reported that the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) values of the fresh onion juices against the test bacteria were low ranging from 3.125%v/v – 25.0% v/v.

Shams-Ghahfarokhi *et al*. (2006) reported that aqueous extracts of onion showed dose dependent protective effect against pathogenic yeast and dermatophytes. The extract inhibits the growth of *Trichophyton rubrum* and *T. mentagrophytes* by affecting their morphology at cellular and subcellular level as it disrupts the cell membranes and other membrane bound structures (Ghahfarokhi *et al.*, 2004). In different studies, onion's protective effect against pathogenic bacteria (*Escherichia coli, Pseudomonas sp.*) and fungi (*Candida, Cryptococcus* and *Malassezia* species.) is in dose dependent manner (Irkin and Arslan, 2010). This antimicrobial activity of onion is also variety dependent as few varieties like green onion have least preventive effect against microbes. Santas *et al*., (2010) had reported that the ethyl acetate sub-fractions of onion showed prevention against microbes which may be due to the presence of flavonoids in these extracts. In another study, it was found that onion inhibits the pro-inflammatory messengers and bacterial growth due to the presence of various organic sulfur compounds (Wilson and Adams, 2007).

The significant growth inhibitions of the test organisms by the plant extracts suggests their possible use in controlling these organisms in disease-causing situations and food spoilage. Furthermore, the easy means of obtaining these extracts especially using water base extraction provides an alternative to antibiotics and artificial preservatives, both of which can be toxic at certain concentrations.

C0NCLUSION

Allium cepa (Onions) and *Allium sativum* (Garlic) have antibacterial properties against common opportunistic bacteria; *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Aqueous extract of Galic was the most effective, revealing its potential efficiency for the treatment of diseases arising from these organisms. The antibacterial profile of these plant extracts underline their recognition and application as medicinal plants.

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