A Pilot Study of the Antibacterial Activity of Garlic (*Allium Sativum*) Against Selected Drug Resistant Organisms

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ABSTRACT

The objective of this research was to determine the antibacterial effects of the aqueous and ethanol extracts of *Allium sativum* (Garlic) against selected drug resistant organisms of *Staphylococcus spp* and *Escherichia coli.* We carried out antibacterial tests on the selected drug resistant organisms using paper disc diffusion by kirby-bauer disc diffusion method. Whiteman filter paper was cut into pieces of 6mm and dipped into different concentrations of the extracts that were serially diluted, allowed for proper diffusion for 30minutes and placed on the pre-inoculated plates of the test organisms, incubated for 24hours after which zone of inhibition diameter (ZID) were measured in MM. The results indicated that 400mg/ml concentration and the control antibiotics(AUG-30mg) were effective against *staphylococcus* spp and *Escherichia coli* for both aqueous and ethanol extracts with zone of inhibition of 15,15,10,14,20,23,10,13 respectively. the 200mg/ml of both aqueous and ethanol extracts was also effective against the test organisms with zones of inhibition diameter of 0,13,13,0 for the resistant strains of Staphylococcus aureus and Escherichia coli respectively.while the concentrations of 100mg/ml and 50mg/ml didn't show any zone of inhibition against the test organisms.the MIC and MBC for Staphylococcus aureus in both extracts is 400mg/ml while MIC for *Escherichia coli* in both extracts is 200mg/ml and MBC IS 400mg/ml. Both the control and different concentrations of 400mg/ml and 200mg/ml of the extracts were active against the selected resistant organisms while some strains were resistant to the extracts especially to the cocentrations of 100mg/ml and 50mg/ml. The result showed that Garlic could be very effective as an antibacterial agent against drug resistant organisms of *Staphylococcus aureus* and *Escherichia coli* mostly at a high concentration dosages and dependent on the type of drug resistant organism.

Keywords: Garlic, Extracts, *Staphylococcus aureus*, *Escherichia coli*, Resistance

INTRODUCTION

Background of the Study

Drug-resistant bacteria have developed novel resistance mechanisms, leading to antimicrobial resistance which continued to pose a danger to our capacity to treat common diseases. Over the last few decades, dangerous antibiotic-resistant microorganisms have become increasingly common. Because of the rise in infectious diseases and pathogenic microorganisms' resistance to chemical drugs over time, as well as the side effects and high treatment costs that chemical and synthetic drugs impose on human societies, the use of medicinal plants of natural origin has become more common in recent years. Diseases transferred to humans through food have

become a big problem in recent years, and the negative side effects of synthetic preservatives added to food have led to skepticism regarding these preservatives (Almeida et al., 2018; Rohr *et al*., 2019). As a result, several researchers have been driven to do follow-up studies to replace these preservatives with herbal and natural substances that produce similar results while having fewer side effects (Subramani *et al*., 2017; Helal et al., 2019; Zhao et al., 2019).Essential oils and plant extracts, which are volatile and aromatic chemicals with good antibacterial activity, are among these components (Cui et al., 2020; Shin et al., 2022).

Garlic, scientifically known as Allium sativum, is a member of the Amaryllidaceae family. This plant is employed to cure infectious diseases in traditional medicine (Ayaz & Alpsoy, 2007; Goncagul & Ayaz, 2010; Daka, 2011). This plant was used to treat a variety of ailments in ancient texts and literature, including heart disease, Garlic (Allium sativum), a popular food spice and flavoring agent, has also been used traditionally to treat various ailments especially bacterial infections for centuries in various cultures around the world. The principal phytochemicals that exhibit antibacterial activity are oil-soluble organosulfur compounds that include allicin, ajoenes, and allyl sulfides. The organosulfur compounds of garlic exhibit a range of antibacterial properties such as bactericidal, antibiofilm, antitoxin, and anti-quorum sensing activity against a wide range of bacteria including multi-drug resistant (MDR) strains. The reactive organosulfur compounds form disulfide bonds with free sulfhydryl groups of enzymes compromise the integrity of the bacterial membrane. The World Health Organization (WHO) has recognized the development of antibiotic resistance as a global health concern and emphasizes antibiotic stewardship along with the urgent need to develop novel antibiotics.

Multiple antibacterial effects of organosulfur compounds provide an excellent framework to develop them into novel antibiotics. Garlic (Allium sativum), belonging to family Liliaceae, mainly the bulb of garlic, has been used as a spice in cooking worldwide especially in Italy and Southeast Asia. More importantly, garlic has been an ingredient in folk and traditional medicine since ancient times (Rivlin, 2001). Garlic is cultivated all over the world with a per-capita consumption of two pounds per year. As per the Food and Agricultural Organization of the United Nations, China and India are first and second, respectively, in average (1961 –2017) garlic production. Health benefits that are associated with the use of garlic are attributed to its anticancer, anti-inflammatory, antifungal, antiviral, and antibacterial activity. Several in vitro, in vivo, and epidemiological studies indicate that garlic exhibits anticancer activity, and the likely mechanism of action is by activating metabolizing enzymes, inhibiting reactive oxygen species, radical scavenging, preventing DNA damage, and tumor inhibition (Cao et al., 2014; Zhang et al., 2019). The immunomodulatory effects of garlic are mediated through its ability to modulate cytokine production as well as activate immune response by stimulating antibody secretion and immune cells (Arreola et al., 2015). Garlic displays anti allergic properties by inhibiting antibody-mediated histamine production and modulates airway allergic response (Kyo et al., 2001; Zare et al., 2008). The anti-inflammatory and anti-arthritic ability of garlic comes from its ability to inhibit NF-κB signaling (Ban et al., 2009). Garlic oil (GO) exhibits antifungal activity against Candida albicans and Penicillium funiculosum by penetration into cells and organelles and causing differential expression of genes that are critical for cellular metabolism (Li et al., 2016). One of the earliest reports of garlic's antibacterial activity was by Small *et al*. (1947) and Stoll and Seebeck (1947). Since then, extensive research has been performed on the antibacterial effects of garlic. The antibacterial activity against various pathogenic and drug-resistant bacteria was tested using crude garlic extracts, garlic powder (GP), garlic extracts using various solvents, GO, and phytochemicals isolated from garlic. The constant and rapid emergence of antimicrobial resistance has been recognized as an alarming threat to human health, which mandates the scientific community to develop novel and effective antibacterial agents (Cheng *et al*., 2016). Garlic compounds exhibit multiple modes of antibacterial activity and have enormous potential to be developed into novel antibacterial agents. Most reviews about garlic discuss the antibacterial activity of garlic as one of its many health benefits diluting importance of garlic compounds as potential antibacterial agents hence this study is particularly interested on the antibacterial activity of garlic against drug resistant strains of *Escherichia coli* and *staphylococcus spp*.

Persistence versus resistance

Before discussing the various aspects of antimicrobial resistance, it would be helpful to distinguish resistance from persistence. If a bacterium is resistant to a certain antimicrobial agent, then all of the daughter cells would also be resistant (unless additional mutations occurred in the meantime). Persistence, however, describes

bacterial cells that are not susceptible to the drug, but do not possess resistance genes. The persistence is undoubtedly due to the fact that some cells in a bacterial population may be in stationary growth phase (dormant); and most antimicrobial agents have no effect on cells that are not actively growing and dividing. These persister cells occur at a rate of around 1% in a culture that is in stationary phase (Wood et al., 2013). Figure 1 shows the difference between persistent and resistant bacterial cells.

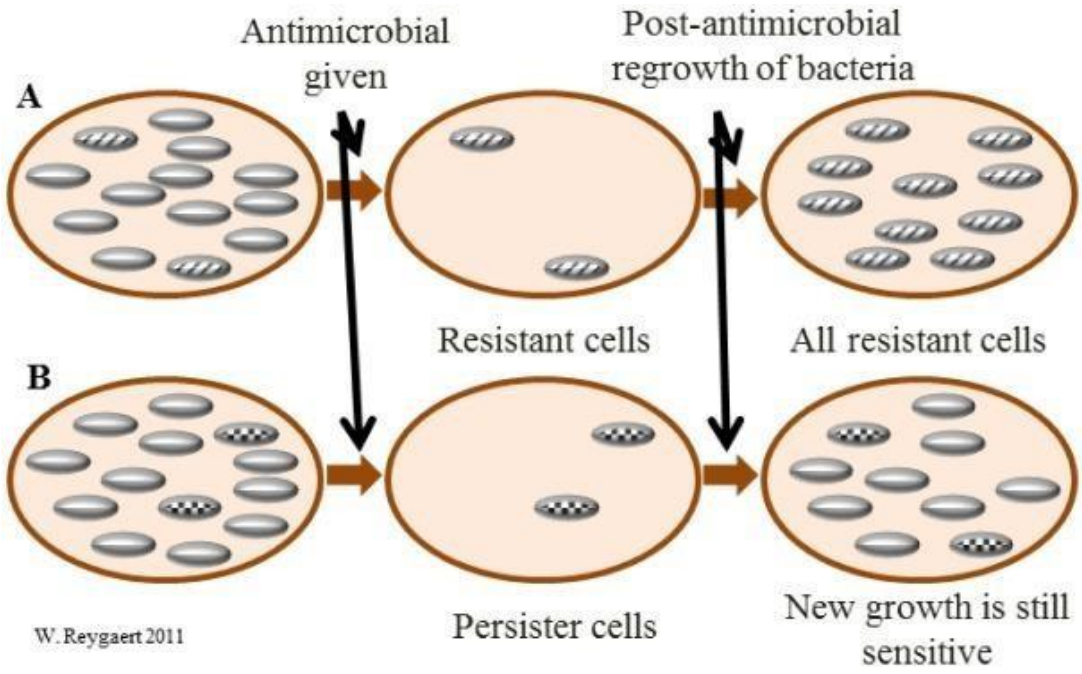

Figure 2.

Resistance vs. persistence. When bacterial cells are exposed to an antimicrobial agent there are two possible scenarios. There may be cells present that are resistant to the antimicrobial agent (A). The non-resistant cells are killed, leaving only the resistant cells. When the resistant cells are regrown, all of the cells in the culture will be resistant. The other possibility is that there may be persister cells (dormant, not resistant) present (B). The non-persister cells are killed, leaving only the persister cells. When the persister cells are regrown, those cells not in a dormant state will still be susceptible to the antimicrobial agent.

Origins of resistance

Bacteria as a group or species are not necessarily uniformly susceptible or resistant to any particular antimicrobial agent. Levels of resistance may vary greatly within related bacterial groups. Susceptibility and resistance are usually measured as a function of minimum inhibitory concentration (MIC), the minimal concentration of drug that will inhibit growth of the bacteria. The susceptibility is actually a range of the average MICs for any given drug across the same bacterial species. If that average MIC for a species is in the resistant part of the range, the species is considered to have intrinsic resistance to that drug. Bacteria may also acquire resistance genes from other related organisms, and the level of resistance will vary depending on the species and the genes acquired (Coculescu, 2009).

Natural resistance

Natural resistance may be intrinsic (always expressed in the species), or induced (the genes are naturally occurring in the bacteria, but are only expressed to resistance levels after exposure to an antibiotic). Intrinsic resistance may be defined as a trait that is shared universally within a bacterial species, is independent of previous antibiotic exposure, and not related to horizontal gene transfer (Martinez, 2014). The most common bacterial mechanisms involved in intrinsic resistance are reduced permeability of the outer membrane (most specifically the lipopolysaccharide, LPS, in gram negative bacteria) and the natural activity of efflux pumps. Multidrug-efflux pumps are also a common mechanism of induced resistance (Cox et al., 2013). Table 2 shows some examples of bacteria with intrinsic antimicrobial resistance.

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Table 2.1 Examples of bacteria with intrinsic resistance.

Organism	Intrinsic resistance
Bacteroides (anaerobes)	aminoglycosides, many β-lactams, quinolones
All gram positives	Aztreonam
Enterococci	aminoglycosides, cephalosporins, lincosamides
Listeria monocytogenes	Cephalosporins
All gram negatives	glycopeptides, lipopeptides
Escherichia coli	Macrolides
Klebsiella spp.	Ampicillin
Serratia marcescens	Macrolides
Pseudomonas aeruginosa	sulfonamides, ampicillin, 1st and 2nd generation cephalosporins, chloramphenicol, tetracycline
Stenotrophomonas maltophilia	aminoglycosides, β-lactams, carbapenems, quinolones
Acinetobacter spp.	ampicillin, glycopeptides

Acquired resistance

Acquisition of genetic material that confers resistance is possible through all of the main routes by which bacteria acquire any genetic material: transformation, transposition, and conjugation (all termed horizontal gene transfer—HGT); plus, the bacteria may experience mutations to its own chromosomal DNA. The acquisition may be temporary or permanent. Plasmid-mediated transmission of resistance genes is the most common route for acquisition of outside genetic material; bacteriophage-borne transmission is fairly rare. Certain bacteria such as Acinetobacter spp. are naturally competent, and therefore capable of acquiring genetic material directly from the outside environment. Internally, insertion sequences and integrins may move genetic material around, and stressors (starvation, UV radiation, chemicals, etc.) on the bacteria are common causes of genetic mutations (substitutions, deletions etc.). Bacteria have an average mutation rate of 1 for every 106 to 109 cell divisions, and most of these mutations will be deleterious to the cell (Coculescu, 2009). Mutations that aid in antimicrobial resistance usually only occur in a few types of genes; those encoding drug targets, those encoding drug transporters, those encoding regulators that control drug transporters, and those encoding antibiotic-modifying enzymes. In addition, many mutations that confer antimicrobial resistance do so at a cost to the organism. For example, in the acquisition of resistance to methicillin in Staphylococcus aureus, the growth rate of the bacteria is significantly decreased.

One huge conundrum of antimicrobial resistance is that the use of these drugs leads to increased resistance. Even the use of low or very low concentrations of antimicrobials (sub-inhibitory) can lead to selection of high-level resistance in successive bacterial generations, may select for bacteria that are hypermutatable strains (increase the mutation rate), may increase the ability to acquire resistance to other antimicrobial agents, and may promote the movement of mobile genetic elements(Blázquez et al.,2012).

Mechanisms of Antimicrobial resistance

Antimicrobial resistance mechanisms fall into four main categories: (WHO, 2014) limiting uptake of a drug (WHO, 2015) modifying a drug target; inactivating a drug; active drug efflux. Intrinsic resistance may make use of limiting uptake, drug inactivation, and drug efflux; acquired resistance mechanisms used may be drug target modification, drug inactivation, and drug efflux. Because of differences in structure, etc., there is variation in the types of mechanisms used by gram negative bacteria versus gram positive bacteria. Gram negative bacteria make use of all four main mechanisms, whereas gram positive bacteria less commonly use limiting the uptake of a drug (don't have an LPS outer membrane), and don't have the capacity for certain types of drug efflux mechanisms (Mahon, 2014). Figure 2 illustrates the general antimicrobial resistance mechanisms.

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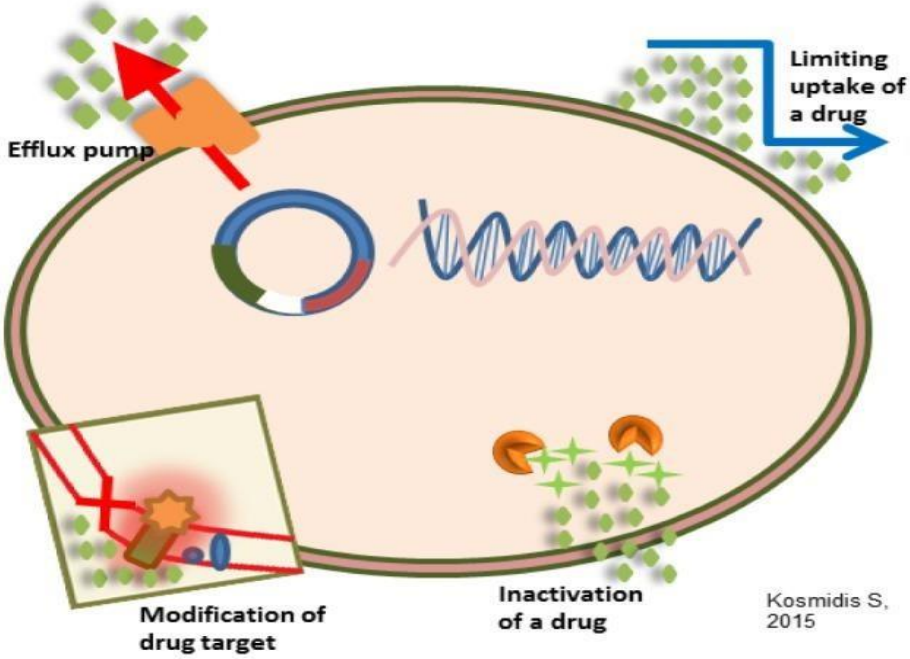

Figure 2.1 General antimicrobial resistance mechanisms

Limiting drug uptake

As already mentioned, there is a natural difference in the ability of bacteria to limit the uptake of antimicrobial agents. The structure and functions of the LPS layer in gram negative bacteria provides a barrier to certain types of molecules. This gives those bacteria innate resistance to certain groups of large antimicrobial agents. The mycobacteria have an outer membrane that has a high lipid content, and so hydrophobic drugs such as rifampicin and the fluoroquinolones have an easier access to the cell, but hydrophilic drugs have limited access (Kumar et al., 2005).

Bacteria that lack a cell wall, such as Mycoplasma and related species, are therefore intrinsically resistant to all drugs that target the cell wall including β-lactams and glycopeptides (Bébéar et al., 2005). Gram positive bacteria do not possess an outer membrane, and restricting drug access is not as prevalent. In the enterococci, the fact that polar molecules have difficulty penetrating the cell wall gives intrinsic resistance to aminoglycosides. Another gram positive bacteria, Staphylococcus aureus, recently has developed resistance to vancomycin. Of the two mechanisms that S. aureus uses against vancomycin, a yet unexplained mechanism allows the bacteria to produce a thickened cell wall which makes it difficult for the drug to enter the cell, and provides an intermediate resistance to vancomycin. These strains are designated as VISA strains (Miller et al., 2014).

In those bacteria with large outer membranes, substances often enter the cell through porin channels. The porin channels in gram negative bacteria generally allow access to hydrophilic molecules (Blair et al., 2014). There are two main ways in which porin changes can limit drug uptake: a decrease in the number of porins present, and mutations that change the selectivity of the porin channel (Kumar et al., 2005). Members of the Enterobacteriaceae are known to become resistant due to reducing the number of porins (and sometime stopping production entirely of certain porins). As a group, these bacteria reduce porin number as a mechanism for resistance to carbapenems. Mutations that cause changes within the porin channel have been seen in E. aerogenes which then become resistant to imipenem and certain cephalosporins, and in Neisseria gonorrhoeae which then become resistant to β-lactams and tetracycline.

Another widely seen phenomenon in bacterial colonization is the formation of a biofilm by a bacterial community. These biofilms may contain a predominant organism (such as by Pseudomonas aeruginosa in the lung), or may consist of a wide variety of organisms, as seen in the biofilm community of normal flora in the gut. For pathogenic organisms, formation of a biofilm protects the bacteria from attack by the host immune system, plus provides protection from antimicrobial agents. The thick, sticky consistency of the biofilm matrix

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which contains polysaccharides, and proteins and DNA from the resident bacteria, makes it difficult for antimicrobial agents to reach the bacteria. Thus, to be effective, much higher concentrations of the drugs are necessary. In addition the bacterial cells in the biofilm tend to be sessile (slow metabolism rate, slow cell division), so antimicrobials that target growing, dividing bacterial cells have little effect. An important observation about biofilms is that it is likely that horizontal transfer of genes is facilitated by the proximity of the bacterial cells. That means that sharing of antimicrobial resistance genes is potentially easier for these bacterial communities (Mah, 2012).

Modification of drug targets

There are multiple components in the bacterial cell that may be targets of antimicrobial agents; and there are just as many targets that may be modified by the bacteria to enable resistance to those drugs. One mechanism of resistance to the β-lactam drugs used almost exclusively by gram positive bacteria is via alterations in the structure and/or number of PBPs (penicillin-binding proteins). PBPs are transpeptidases involved in the construction of peptidoglycan in the cell wall. A change in the number (increase in PBPs that have a decrease in drug binding ability, or decrease in PBPs with normal drug binding) of PBPs impacts the amount of drug that can bind to that target. A change in structure (e.g. PBP2a in S. aureus by acquisition of the mecA gene) may decrease the ability of the drug to bind, or totally inhibit drug binding (Beceiro et al., 2013).

The glycopeptides (e.g. vancomycin) also work by inhibiting cell wall synthesis, and lipopeptides (e.g. daptomycin) work by depolarizing the cell membrane. Gram negative bacteria (thick LPS layer) have intrinsic resistance to these drugs. Resistance to vancomycin has become a major issue in the enterococci (VRE—vancomycin-resistant enterococci) and in Staphylococcus aureus (MRSA). Resistance is mediated through acquisition of van genes which results in changes in the structure of peptidoglycan precursors that cause a decrease in the binding ability of vancomycin (Beceiro et al., 2013). Daptomycin requires the presence of calcium for binding. Mutations in genes (e.g. mprF) change the charge of the cell membrane surface to positive, inhibiting the binding of calcium, and therefore, daptomycin.

Resistance to drugs that target the ribosomal subunits may occur via ribosomal mutation (aminoglycosides, oxazolidinones), ribosomal subunit methylation (aminoglycosides, macrolides—gram positive bacteria, oxazolidinones, streptogramins) most commonly involving erm genes, or ribosomal protection (tetracyclines). These mechanisms interfere with the ability of the drug to bind to the ribosome. The level of drug interference varies greatly among these mechanisms (Kumar et al., 2013).

For drugs that target nucleic acid synthesis (fluoroquinolones), resistance is via modifications in DNA gyrase (gram negative bacteria—e.g. gyrA) or topoisomerase IV (gram positive bacteria—e.g. grlA). These mutations cause changes in the structure of gyrase and topoisomerase which decrease or eliminate the ability of the drug to bind to these components (Redgrave et al., 2014).

For the drugs that inhibit metabolic pathways, resistance is via mutations in enzymes (DHPS—dihydropteroate synthase, DHFR—dihydrofolate reductase) involved in the folate biosynthesis pathway and/or overproduction of resistant DHPS and DHFR enzymes (sulfonamides—DHPS, trimethoprim—DHFR). The sulfonamides and trimethoprim bind to their respective enzymes due to their being structural analogs of the natural substrates (sulfonamides—p-amino-benzoic acid, trimethoprim—dihydrofolate). The action of these drugs is through competitive inhibition by binding in the active site of the enzymes. Mutations in these enzymes are most often located in or near the active site, and resulting structural changes in the enzyme interfere with drug binding while still allowing the natural substrate to bind.

Drug inactivation

There are two main ways in which bacteria inactivate drugs; by actual degradation of the drug, or by transfer of a chemical group to the drug. The β-lactamases are a very large group of drug hydrolyzing enzymes. Another drug that can be inactivated by hydrolyzation is tetracycline, via the tetX gene (Blair et al., 2015).

Drug inactivation by transfer of a chemical group to the drug most commonly uses transfer of acetyl, phosphoryl, and adenyl groups. There are a large number of transferases that have been identified. Acetylation

is the most diversely used mechanism, and is known to be used against the aminoglycosides, chloramphenicol, the streptogramins, and the fluoroquinolones. Phosphorylation and adenylation are known to be used primarily against the aminoglycosides (Schwarz et al., 2004).

Active Phytochemicals of Garlic

Most of the health benefits of garlic are attributed to a myriad of cysteine-derived sulfur-containing organic compounds present in garlic (extensively reviewed in Fenwick and Hanley, 1985a, b, c). The organosulfur compounds of intact garlic clove greatly differ from that present in garlic juice obtained after crushing garlic. The intact garlic mainly contains non-volatile γ-glutamyl-S-alk(en)yl-L-cysteines, namely, γ-glutamyl-S-allyl-L-cysteine, γ-glutamyl-S-trans-1-propenyl-L-cysteine, and S-alk(en)yl-L-cysteine sulfoxides such as S-allyl-L-cysteine sulfoxide (alliin), S-(trans-1-propenyl)- L-cysteine sulfoxide (isoalliin), and S-methyl-L- cysteine sulfoxide (methiin) with a small amount of S-allyl cysteine (SAC) (Block, 1992). Crushing or cutting garlic cloves releases allinase enzyme sequestered in the vacuoles, which encounters cytosolic alliin to convert it into an array of thoisulfinates of which the most prominent is allicin. The highly reactive, unstable, and volatile allicin decomposes to yield a large number of sulfides such as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), methyl allyl disulfide (MADS), methyl allyl sulfide, ajoene, and vinyl dithiins (2 -vinyl-1,3-dithiin, 3-vinyl-1,2-dithiin)(Brodnitz et al., 1971). The sulfides are oil-soluble compounds that are responsible for the characteristic garlic odor and flavor. Allicin exhibits excellent in vitro antibacterial activity, which resulted in a huge number of studies to evaluate the potential of allicin and oil-soluble organosulfur compounds of garlic as antibacterial agents (Cavallito and Bailey, 1944a). A large body of literature supports the antibacterial potential of garlic organosulfides. The organosulfur compounds present in the aqueous and alcoholic extract of garlic include S-allyl cysteine (SAC), S-allylmercapto-L-cysteine (SAMC), and S-methyl cysteine. The compounds are non-volatile, non-odiferous, and stable compounds compared to volatile organosulfides. Most health benefits of garlic are largely attributed to these organosulfur compounds present in garlic. However, garlic organosulfur compounds are very unstable with low bioavailability and the presence of these compounds depends on the processing of the garlic during the preparation of garlic supplements (Amagase et al., 2001; Amagase, 2006).

The main antibacterial organosulfur compounds of garlic are allicin, ajoene, and various aliphatic sulfides. The extraction procedure results in concentrating a particular compound rather than providing a pure compound. Extraction of garlic with water or ethanol and concentrating the extract will provide an allicin-rich product. It was noticed that yield with ethanol is better compared to water (Fujisawa et al., 2008). However, extraction of concentrated ethanolic distillate with organic solvent yields a highly concentrated and pure allicin product (Cavallito and Bailey, 1944a; Ratnakar and Murthy, 1995). Later, it was reported that extraction using acetone will yield higher allicin compared to ethanol (Canizares et al., 2004a). Recently, salting-out extraction using ethanol and ammonium sulfate result in effective extraction of allicin (Li et al., 2017). Oil-macerated garlic extract has a very high proportion of ajoene along with other thiosulfinates (Yoshida et al., 1998). Steam distillation of garlic yields GO, which mainly consists of various aliphatic sulfides (Avato et al., 2000). The components of both oil-macerated garlic extract and GO can be separated using chromatographic and distillation techniques (Casella et al., 2013).

PubMed search of “Garlic antibacterial” yields more than 350 research papers. This large body of literature comprises research papers that investigated the antibacterial activity of crude preparation of garlic, various extracts of garlic, and individual organosulfur compounds of garlic against various bacteria including MDR bacteria. It provides a list of in vitro antibacterial activity of various garlic products and compounds against different bacteria. Some of the early research that reported the antibacterial activity of garlic against a wide variety of bacteria has been summarized by Adetumbi and Lau (1983). The present review provides a comprehensive summary of this large body of research.MRSA strains (Cutler and Wilson, 2004). Similarly, aqueous allicin extract and cream demonstrated anti-Lancefield group B streptococci clinical isolate using in vitro assays (Cutler et al., 2009). A comparative in vitro study of antibacterial activity against S. aureus and E. coli activity of FGE, allicin, and clinically used antibiotics was performed. The results of the study indicated that fresh garlic was more potent against S. aureus compared to allicin and not much difference in activity was noticed against E. coli while both bacteria were more sensitive to antibiotics than garlic extract or allicin (Fujisawa et al., 2009). The administration of allicin to rainbow trout through its diet almost eliminated

mortality when infected with Aeromonas hydrophila, a fish pathogen. In addition, in vitro studies also indicated that this bacterium was sensitive to allicin (Nya et al., 2010). It was found that A. actinomycetemcomitans was sensitive to allicin, and this activity disappeared upon heating, indicating that allicin is thermolabile (Velliyagounder et al., 2012). Although an in vitro assay found that C. jejuni was sensitive to allicin, in vivo studies indicated that allicin had no significant effect on colonization of C. jejuni in broilers. The possible explanation for this could be that the presence of mucus inhibited the activity of allicin in vitro (Robyn et al., 2013). In addition to AGE, allicin also exhibits dose-dependent antibacterial activity against Bcc (Wallock-Richards et al., 2014). In an interesting study, allicin vapors were able to exhibit bactericidal activity against MDR lung pathogenic bacteria such as P. aeruginosa and Streptococcus pyogenes (Reiter et al., 2017). It was found that the active ingredient of Bald's eyeslave, an Anglo-Saxon medical remedy made up of garlic, onions, bovine bile, and brass effective against S. aureus and P. aeruginosa, was allicin (Fuchs et al., 2018). Allicin is the most potent antibacterial organosulfur compound found in garlic. The higher activity is thought to be due to the highly reactive sulfoxide group of allicin. However, the stability and solubility of allicin are the challenges in its clinical use. Animal studies highlight the reduced bioavailability and toxicity associated with allicin administration (Amagase et al., 2001).

Gupta and Ravishankar, 2005 hinted that the causative agent of gastric ulcers, Helicobacter pylori (standard strains types and clinical isolates), was found to be sensitive to GP and 1,000 μg/ml of GP inactivated H. pylori at 6 h in a time course viability assay (O'Gara et al., 2000). Allicin-rich crude extract exhibited better antibacterial activity against Mycobacterium phlei, Mycobacterium smegmatis, and Mycobacterium tuberculosis compared to isoniazid and ethambutol. Also, disk diffusion assay with allicin-rich extract exhibited significant activity against MRSA (Viswanathan et al., 2014). Another study also found that FGE was effective against MDR strains of E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens, and MRSA in both in vitro and in vivo assays (Farrag et al., 2019).

Antibacterial Activity of Garlic Fresh Extract and Powder

Garlic is one of the popular spices added to food to enhance the flavor, and it has been used in different cultures and traditions around the world to treat bacterial infections for centuries. Several studies have evaluated the antibacterial activity of various garlic preparations such as crude or fresh garlic extract (FGE), and garlic paste. The antibacterial activity of garlic paste and FGE against commensal and pathogen enteric bacteria such as Escherichia coli, E. coli O157:H7, Salmonella species, Shigella species, Vibrio species, Campylobacter species, Listeria monocytogenes, Enterobacter, and Enterococcus species, Lactobacillus acidophilus, Staphylococcus aureus, Streptococcus species, and Clostridium difficile has been reported by various laboratories (Johnson and Vaughn, 1969; Kumar and Berwal, 1998; Ross et al., 2001; Gupta and Ravishankar, 2005; Vuddhakul et al., 2007; Lu et al., 2011b; Jain et al., 2015a; Roshan et al., 2017).

These studies suggest that garlic consumption could help in preventing food poisoning. In addition, various studies have evaluated the impact of garlic and its organosulfur compounds on the gut microbiome. Garlic was found to positively influence the gut microbiome and protect the gut microbiome damage from high-fat diet (Chen et al., 2019). Supplementing feed of farrowing sows and European bass with GO decreased pathogenic microbes from the gut microbiome (Rimoldi et al., 2020; Satora et al., 2020). Allicin treatment prevented high carnitine diet-induced dysbiosis to lower the atherosclerosis risk factor trimethylamine N-oxide that is produced by the gut microbiome (Wu W. K. et al., 2015). Oral administration of alliin, precursor of allicin, to rats resulted in decreasing the relative abundance of only Allobaculum genus in the cecum (Zhang et al., 2019). The gut microbiome was altered upon intragastric administration of DADS of rat, a low dose of DADS decreased Bacteroidetes phyla but increased Firmicutes phyla bacteria (Yang et al., 2019). Oral administration of propyl propane thiosulfonate restored the richness and evenness of gut microbiome lost due to dextran sodium sulfate-induced colitis in mice (Vezza et al., 2019).

A small-scale clinical trial, aged garlic extract supplementation for 3 months increases the richness and diversity of the gut microbiome with increase in Lactobacillus and Clostridium species (Ried et al., 2018). All the studies indicate that garlic and its compounds have a positive effect on gut microbiome composition and richness. However, the mechanistic details still need to be investigated. From recent study, FGE exhibited activity against MDR Shiga-toxin producing E. coli (STEC) isolates from clinical and food samples

(Bhatwalkar et al., 2019). In addition to antibacterial activity, garlic crude and aqueous extract exhibited anti-adherent activity against the standard strain type of Streptococcus mutans (Jain et al., 2015a). The data suggest that garlic could be used to preserve food and prevent foodborne infections. However, the antibacterial activity was dramatically decreased when experiments were performed with buffered peptone water and ground beef, suggesting that further research is required to utilize garlic as a food/meat-preserving agent (Gupta and Ravishankar, 2005). The causative agent of gastric ulcers, Helicobacter pylori (standard strains types and clinical isolates), was found to be sensitive to GP and 1,000 μg/ml of GP inactivated H. pylori at 6 h in a time course viability assay (O'Gara et al., 2000). Allicin-rich crude extract exhibited better antibacterial activity against Mycobacterium phlei, Mycobacterium smegmatis, and Mycobacterium tuberculosis compared to isoniazid and ethambutol. Also, disk diffusion assay with allicin-rich extract exhibited significant activity against MRSA (Viswanathan et al., 2014). Another study also found that FGE was effective against MDR strains of E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens, and MRSA in both in vitro and in vivo assays (Farrag et al., 2019).

Antibacterial Activity of Garlic Aqueous Extract

There are several reports of antibacterial activity of aqueous garlic extract (AGE) against a variety of bacteria. In vitro assay with AGE (10%) showed complete inhibition of Bacillus cereus and the activity varies upon the storage conditions and heat treatment of the aqueous extract (Saleem and Al-Delaimy, 1982). AGE exhibited in vitro antibacterial activity against various pathogenic bacteria including Shigella and Salmonella species and enterotoxigenic E. coli (Arora and Kaur, 1999). In addition, AGE fully cured the rabbits that were challenged with Sh. flexneri Y by completely clearing them of bacteria with no significant side effects (Chowdhury et al., 1991).Supporting the results obtained with GP, in vitro assays indicated that H. pylori is sensitive to AGE, and the sensitivity was more compared to S. aureus (Cellini et al., 1996; Sivam, 2001). In vitro antibacterial assays report that AGE is effective against various Gram-positive and Gram-negative oral bacteria, which include periodontal pathogenic bacteria Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and S. mutans (Bakri and Douglas, 2005; Fani et al., 2007; Velliyagounder et al., 2012). Different studies reported that AGE exhibited activity against a large variety of Gram-positive and Gram-positive pathogenic bacteria including MDR strains and isolates such as MDR M. tuberculosis showing not only the effectiveness of garlic against drug-resistant bacteria but also its broad spectrum (Iwalokun et al., 2004; Gupta et al., 2010; Gull et al., 2012; Meriga et al., 2012). In an interesting study, it was found that counts of S. aureus in hamburger upon addition of AGE reduced in a dose-dependent manner during storage for different time points in the fridge and freezer, supporting the idea of using garlic for meat preservation (Mozaffari Nejad et al., 2014).

To compare the antibacterial activity of various garlic health products, aqueous extracts of different products that included GP, GO, gelatinous GP suspension, aged garlic extract, and gelatinous suspension of aged garlic extract were prepared along with fresh garlic. All the extracts exhibited activity against Neisseria gonorrhoeae, S. aureus, and Enterococcus faecalis. The activity was correlated to the amount of fresh garlic constituents, namely, allicin and SAC, present in the products (Ruddock et al., 2005). The Burkholderia cepacia complex (Bcc) consists of 17 different species of soil bacteria that are pathogenic to allium species. These bacteria cause life-threatening lung infections in patients suffering from cystic fibrosis. AGE exhibited activity against Bcc, and this activity correlated with the allicin content of the extract (Wallock-Richards et al., 2014). In a recent study, non-aged and aged garlic cloves were pressed to remove their juices, dried, and powdered before extracted with water, ethanol, and chloroform. All three extracts of aged garlic exhibited antibacterial activity while only chloroform extract of non-aged garlic had activity against B. cereus (Jang et al., 2018). All these studies indicate that allicin is the main phytochemical responsible for the antibacterial activity of AGE. Although the ethanol extract of garlic also has allicin, AGE is more effective due to the presence of other antibacterial chemicals, which might result in a synergistic or additive effect.

Antibacterial Activity of Garlic Ethanolic Extract

HPLC analysis of ethanolic extract of garlic (EGE) revealed that it contains various thoisulfinates, the major one being allicin. The anti-H. Pylori activity of this extract decreased with the decrease in the concentration of allicin. Furthermore, it was seen that the maturation of garlic increases the allicin yield and extract with acetone yielding a higher percentage of allicin compared to ethanol (Canizares et al., 2004a, b). In vitro studies

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have reported that EGE was found to show antibacterial activity against various pathogenic bacteria including MDR bacteria, MDR M. tuberculosis isolates, and vancomycin-resistant S. aureus (VRSA) isolates (Hannan et al., 2011; Karuppiah and Rajaram, 2012; Snowden et al., 2014; Liaqat et al., 2015). The antibacterial and antiadherence activity of organic solvent (chloroform, acetone, and ethanol) extracts of garlic was least compared to crude and aqueous extract against S. mutans (Jain et al., 2015a). The leaves of wild garlic (Allium ursinum subsp. ucrainicum) found in Serbia were extracted with 70 and 96% ethanol and 80% and absolute methanol, and the S-alk(en)ylcysteines (alliin, isoalliin, and methiin) content of the extracts was analyzed using NMR studies. The extracts exhibited some degree of antibacterial activity against test enteropathogenic bacterial strains with Salmonella enteritidis being the most sensitive. The tested bacteria were more sensitive to ethanolic extract compared to other extracts (Pavlovic et al., 2017). However, the study should have determined the amount of allicin in the extracts for better interpretation of the results instead of alliin, which is a precursor of allicin. Ethanolic (30%) extract of fermented black garlic exhibited antibacterial activity against 11 bacterial strains that cause oral diseases. Short and long incubation with this extract inhibited the growth of more than 90% of salivary bacteria (Vlachojannis et al., 2018). Water extract of the Toluene extract of garlic has been reported to decrease the mortality of Caenorhabditis elegans from P. aeruginosa infections (Rasmussen et al., 2005) and clear the lungs of mice of P. aeruginosa by modulating inflammation (Bjarnsholt et al., 2005).

Allicin along with other thoisulfinates present in EGE seems to be responsible for its antibacterial activity. Other than ethanol and methanol extract, the chloroform extract of both aged and non-aged garlic exhibited activity against B. cereus by disk diffusion assay (Jang et al., 2018). The hexane extract of solid waste of the GO extraction process exhibited activity against various bacteria including S. aureus and MRSA. DASs present in the extract were responsible for this activity (Nakamoto et al., 2020).

Antibacterial Activity of Garlic Oil

Garlic oil is obtained by steam distillation of macerated or mashed garlic. Reverse-phase high-performance liquid chromatography (HPLC) studies have determined that the GO consists of a large variety of diallyl sulphides and other sulfides (O'Gara et al., 2000; Kim et al., 2004). A recent study has performed an exhaustive analysis of the content of GO and reported that the majority of GO is composed of diallyl and allyl methyl sulfides (Mnayer et al., 2014). The anti-mycobacterium effect of GO was demonstrated using in vitro and in vivo studies (Jain, 1998; Viswanathan et al., 2014). The anti-H. Pylori effect of GO was many folds greater than that of GP. This could be because allicin is the only antibacterial thiosulfinate found in GP whereas GO has many organosulfides. The time course viability studies showed concentration-dependent inhibition of H. pylori by GO with 64 µg/ml resulting in complete inhibition in 4.5 h (O'Gara et al., 2000). However, two independent clinical studies indicated that administration of garlic GO was unable to ameliorate the H. pylori infection (Graham et al., 1999; Aydin et al., 2000). GO has been reported to exhibit antibacterial activity against 14 enteric pathogens and 11 commensal enteric bacteria with commensal bacteria being more sensitive. In time course viability studies, the inhibition of Enterobacter aerogenes growth increased with an increase in the concentration of GO, and complete killing was noticed at 22 mg/ml in 1 h (Ross et al., 2001). In another study, different GOs with varying percentages of DDS and DTS along with pure DDS were tested against Gram-positive (S. aureus and Bacillus subtilis) and Gram-negative (E. coli and P. aeruginosa). The antibacterial activity was not significant; however, the little activity that was exhibited was found with GO with a higher percentage of DDS. Interestingly, pure DDS showed little activity against only selected tested bacteria (Avato et al., 2000). However, disk diffusion assay found GO to be effective against S. aureus, E. coli, P. aeruginosa, B. subtilis, and MRSA (Casella et al., 2013; Viswanathan et al., 2014). An in vitro study tested the activity of GO against 40 S. aureus and 60 MRSA isolates and found that GO was more effective against S. aureus compared to MRSA, although this activity was significantly less than standard antibiotics (Tsao S.M. and Yin, 2001). Another study by the same group has reported that GO is effective against 237 clinical isolates of P. aeruginosa and K. pneumoniae, which also included drug-resistant strains. The minimum inhibitory concentration (MIC) values for P. aeruginosa were smaller compared to K. pneumoniae, and four times MIC of GO eliminated P. aeruginosa and K. pneumoniae in 16 and 24 h, respectively, in kill curve assays (Tsao S. and Yin, 2001). However, weak antibacterial activity of GO against six different bacteria has been reported using in vitro assays (Kim et al., 2004). Other bacteria that were reported to be sensitive to GO are Salmonella typhi, L. monocytogenes, and Campylobacter jejuni (Robyn et al., 2013; Mnayer et al., 2014). The discrepancy in the

antibacterial activity of GO among various in vitro studies could be due to the solubility and volatile nature of GO.

Aim of the study

The aim of this study was to determine the antibacterial effects of the ethanolic and aqueous extracts of garlic against selected drug resistant strains of *staphylococcus spp* and *Escherichia coli.*

Specific Objectives

A. To collect and identify the selected drug resistant strains of *Staphylococcus spp* and *Escherichia coli.*
B. To carry out the aqueous and ethanol extraction of Ginger
C. To determine the antibacterial effects of the aqueous and ethanol extracts of Garlic on the selected drug resistant strains.
D. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the selected drug resistant strains of *Staphylococcus spp and Escherichia coli*

Scope of the Study

This study involved the collection of selected drug resistant strains of *Staphylococcus spp* and Escherichia coli from Romic hospital Afikpo,aqueous and ethanol extraction of Garlic, determination of the antibacterial effects of the extracts on the selected drug resistant strains of Staphylococcus and Escherichia coli, determination of their MIC and MBC.

Justification of the Study

The rate of Multi-drug resistance of bacteria against most antibiotics used for treatment of diseases is quite alarming. Antibiotics resistance has become a global challenge putting serious pressure on the available antibiotics and increasing the cost of health care and most time leads to serious morbidity and mortality. This is caused by over use of drugs, abuse, indiscriminate use of and prescription of antibiotics by clinicians, quacks and patients. This has given room to exploit opportunities to tackle this menance including the determination of the antibacterial effects of most herbal extracts against some drug resistant organisms.

Significance of the Study

The study will provide a data and base-line study on the antibacterial potency of garlic on multi-drug resistant organisms as well as enhance the use of the plant traditionally and in modern medicine in development of potent antibiotics.

MATERIALS AND METHODS

Chemicals

The following chemicals were used: hydrogen peroxide (Damazo Nig.), crystal violet, (Afis Biochemicals Nig), Ethanol (Mark, England) Garlic, Distilled water, and aqueous extracts of garlic.

Media

The culture media used includes: Mannitol salt agar, MacConkey agar and Muller-Hinton agar (Oxoid Ltd, Basingstoke, Hampshire, England), Nutrient agar (Oxide Ltd., UK).

Equipment

The following equipment were used: refrigerator, (Harier thermocool Nig), Incubator, (Kankoid Germany), autoclave, hot air oven (Culfexmed, England), laminar flow chamber (Kankoid Germany) syringes, foil spatula, weighing balance, petri-dishes, masking tape, hand gloves, Bunsen burner, tripod stand, wire guaze,

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wire loop, gas cylinder, pressure pot, measuring cylinder, test tube, forceps, swab stick, microscope, slide, meter ruler (Graduated in mm).

Antibiotics Disc: The antibiotics disc used was Augmentin (30mg).

Extracts: Aqueous and ethanol extracts of Garlic at different concentrations of 400mg/ml,200mg/ml,100mg/ml and 50mg/ml.

Collection and Preparation of the Aqueous and Ethanol Ginger extracts

The Garlic bulbs were bought at the local Eke market, Afikpo, transported directly to microbiology laboratory unit, Akanu Ibiam Federal Polytechnic Unwana; Peeled, washed, choped into pieces and pounded with sterilized mortar, weighed 50g and dissolved into 200ml of both distilled water and 95% ethanol respectively, stored for 72 hours, shaked intermittently for proper dissolution, was filtered using cheese cloth, weighed with a yield of 2g ie 4% yield and reconstituted with 5ml of DMSO to get 0.4g/ml multiplied by 1000 to get 400mg/ml concentration which was further diluted serially in two folds to get 200mg/ml,100mg/ml and 50mg/ml concentrations respectively.

Collection, Identification and standardization of the test organisms

The resistant strains of both *Staphylococcus spp* and *E.coli* were collected from the laboratory unit of Romic hospital Afikpo,transported in a nutrient broth media directly to the microbiology laboratory of Akanu Ibiam Federal Polytechnic Unwana for identification and characterization through cultural and biochemical methods and were standardized to 0.5 mcfarland standard for antibacterial assays.

Media Preparation and Material Sterilisation

The media such as macConkey and mannitol salt agar were prepared according to manufacturer's instructions, sterilized with an autoclave at 121^0c for 15minutes,was asepticaally pourd into petri-dishes and allowed to cool before streaking to obtain pure culture of the isolates

Preparation of Mueller-Hinton Agar: 3.8g of Mueller-Hinton agar was weighed and dissolved in 100ml of distilled water, autoclaved for 15mins, 121 0c and was allowed to cool to the temperature of 40^0c, 20ml of the agar was aseptically poured into different petri-dishes for antibacterial assay.

Determination of the Antibacterial Activity of the Extract

The extracts were serially diluted to obtain 1.0%, 0.5%, 0.25%, and 0.125% solutions in sterile test tubes. Sterilized 9mm filter paper disc soaked in the diluted extracts were placed on the plates and incubated for 24hours at room temperature. The plates were examined for clear zones of inhibition. Presence of zones of inhibition indicated activity. The zones were measured in mm using a meter rule while the MIC and MBC were also determined accordingly.

Determination of the Minimum Inhibitory Concentrations (MIC)

The MIC was determined using tube dilution technique according to (Cheesebrough, 200).

The MIC was determined by taking 2ml of the concentrations of the extracts that inhibited the growth of the resistant strains and mixing it with 2ml of the nutrient broth containing 0.1ml of standardized inoculum of 0.5 Mcfarland standard, the tubes were incubated for 24hrs at 37^0c.it has a tube containing broth and inoculum without the extract as the control. The lowest concentration that showed no visible turbidity (inhibited microbial growth) is regarded as the MIC.

Determination of the MBC

Sterile Mueller hinton agar plates were incubated with samples from each of the test tubes that showed no visible growth from the MIC test. The plates were then incubated at 37^0c for 24hrs. The lowest concentration of the extract yielding no growth was recorded as the minimum bactericidal concentration (MBC).

RESULTS

The results from the antibacterial evaluation studies, minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) were summerised in the tables below:

Table 4.0 Zone of inhibition diameter of the aqueous and ethanol extracts of Garlic

	Aqueous extract		Ethanol Extract	
Concentration(mg/ml)/organisms	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
400	15	10	15	14
200	0	13	13	0
100	0	0	0	0
50	0	0	0	0
Control(AUG-30mg/ml)	20	23	21	13

Table 4.1: Resistance and Susceptibility potentials of the test organisms

Concentration(mg/ml)/organisms	Aqaous extract		Ethanol extract	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*
400	S	S	S	S
200	R	S	S	R
100	R	R	S	R
50	R	R	R	R
Control	S	S	S	S

KEY: R = Resistance, S = susceptible

Table 4.2 : Descriptive analyis for the zone of inhibition for different doses of ethanolic extract of *Allum sativa* (EEAS)

		N	Mean	Std. Deviation	Std. Error
E. coli	Control	2	13.0000	.21213	.15000
	50mg/ml	2	.0000	.00000	.00000
	100mg/ml	2	.0000	.00000	.00000
	200mg/ml	2	.0000	.00000	.00000
	400mg/ml	2	14.0000	.14142	.10000
	Total	10	5.4000	6.97985	2.20722
Staph	Control	2	21.0000	1.41421	1.00000
	50mg/ml	2	.0000	.00000	.00000
	100mg/ml	2	.0000	.00000	.00000
	200mg/ml	2	13.0000	.02828	.02000
	400mg/ml	2	15.0000	.07071	.05000
	Total	10	9.8000	8.89198	2.81189

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Table 4.3:ANOVA table (Post Hoc Tests)

Multiple Comparisons					
Dunnett t (2-sided)[a]					
Dependent Variable	(I) Dosages	(J) Dosages	Mean Difference (I-J)	Std. Error	Sig.
E. coli	50mg/ml	Control	-13.00000*	.11402	.000
	100mg/ml	Control	-13.00000*	.11402	.000
	200mg/ml	Control	-13.00000*	.11402	.000
	400mg/ml	Control	1.00000*	.11402	.001
Staph	50mg/ml	Control	-21.00000*	.63337	.000
	100mg/ml	Control	-21.00000*	.63337	.000
	200mg/ml	Control	-8.00000*	.63337	.000
	400mg/ml	Control	-6.00000*	.63337	.001
*. The mean difference is significant at the 0.05 level.					
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.					

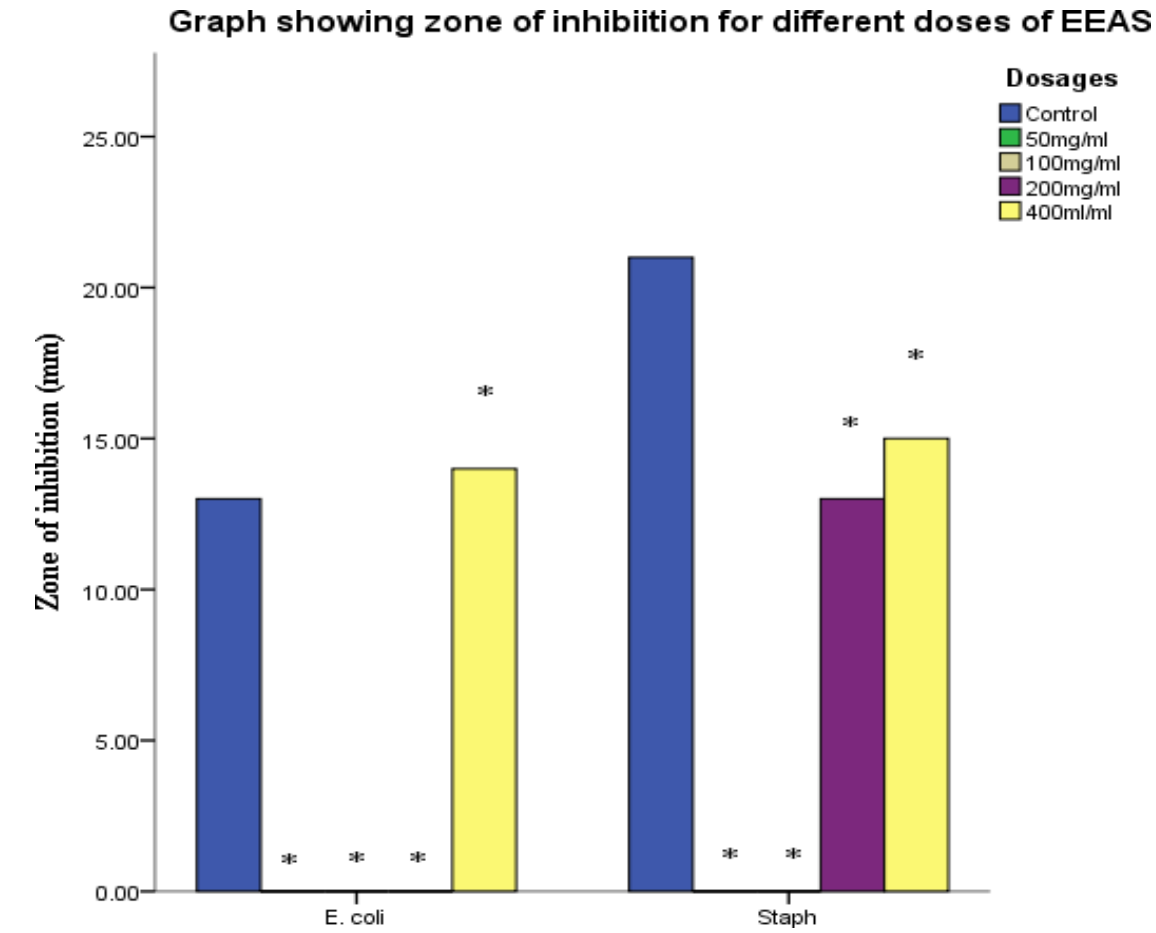

Figure 2.0: Graph showing zone of inhibition for different doses of EEAS

Values are expressed as mean ± SEM, n = 2.

* = significantly different from control at $P < .0$

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Table 4.4 Descriptive analysis for the zone of inhibition for different doses of aqueous extract of *Allum sativa* (AEAS)

		N	Mean	Std. Deviation	Std. Error
`E. coli	Control	2	23.0000	.70711	.50000
	50mg/ml	2	.0000	.00000	.00000
	100mg/ml	2	.0000	.00000	.00000
	200mg/ml	2	13.0000	.07071	.05000
	400mg/ml	2	10.0000	.02828	.02000
	Total	10	9.2000	9.12935	2.88695
Staph	Control	2	20.0000	1.41421	1.00000
	50mg/ml	2	.0000	.00000	.00000
	100mg/ml	2	.0000	.00000	.00000
	200mg/ml	2	.0000	.00000	.00000
	400mg/ml	2	15.0000	.70711	.50000
	Total	10	7.0000	9.20447	2.91071

Table 4.5 ANOVA table (Post Hoc Tests)

Multiple Comparisons					
Dunnett t (2-sided)[a]					
Dependent Variable	(I) Dosages	(J) Dosages	Mean Difference (I-J)	Std. Error	Sig.
E. coli	50mg/ml	Control	-23.00000*	.31806	.000
	100mg/ml	Control	-23.00000*	.31806	.000
	200mg/ml	Control	-10.00000*	.31806	.000
	400mg/ml	Control	-13.00000*	.31806	.000
Staph	50mg/ml	Control	-20.00000*	.70711	.000
	100mg/ml	Control	-20.00000*	.70711	.000
	200mg/ml	Control	-20.00000*	.70711	.000
	400mg/ml	Control	-5.00000*	.70711	.003
*. The mean difference is significant at the 0.05 level.					
a. Dunnett t-tests treat one group as a control, and compare all other groups against it.					

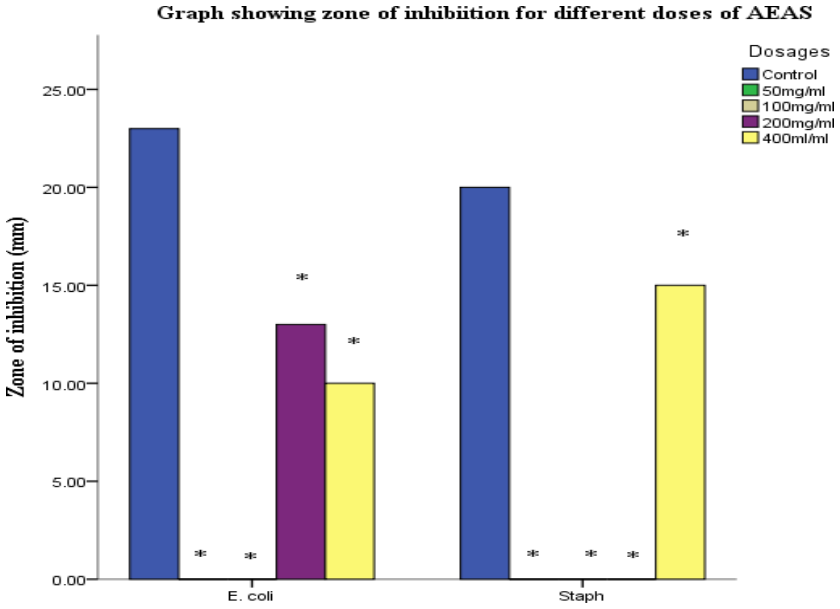

Figure 3.0: Graph showing zone of Inhibition of different doses of AEAS

Values are expressed as mean ± SEM, n = 2.

* = significantly different from control at $P < 0.05$

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DISCUSSION

From table 4.0,4.1,4.2,the results indicated that 400mg/ml concentration and the control antibiotics(AUG-30mg) were effective against resistant strains of *staphylococcus* spp and *Escherichia coli* for both aqueous and ethanol extracts with zone of inhibition of 15,15,10,14,20,23,10,13 respectively.so 400mg/ml is the highest concentration of the extract that showed activity against the resistant strains as seen from table 4.0; the 200mg/ml of both aqueous and ethanol extracts was also effective against the test organisms with zones of inhibition diameter of 0,13,13,0 for the resistant strains of Staphylococcus aureus and Escherichia coli respectively.while the concentrations of 100mg/ml and 50mg/ml didn't show any zone of inhibition against the test organisms.the MIC and MBC for *Staphylococcus aureus* in both extracts is 400mg/ml while MIC for *Escherichia coli* in both extracts is 200mg/ml and MBC IS 400mg/ml. Both the control and different concentrations of 400mg/ml and 200mg/ml of the extracts were active against the selected resistant organisms while some strains were resistant to the extracts especially to the cocentrations of 100mg/ml and 50mg/ml. The result showed that Garlic could be very effective as an antibacterial agent against drug resistant organisms of *Staphylococcus aureus* and *Escherichia coli* mostly at a high concentration dosages and dependent on the type of drug resistant organism. Our results coroborates the work of Emmanuel et al.,2021 where different concentrations of Ginger and garlic were active against different clinical isolates of Escherichia coli,staphylococcus spp,pseudomonas spp.

It was equally interesting to note that our research was in accordance to the findings of Chandarana et al., 2005 where the extracts of ginger and garlic were effective against both gram positive and negative bacteria,though in our case it was against drug resistant strains of both gram negative and positive isolates.

The results of our research is also simmillar to the results of a comparative in vitro study of antibacterial activity of fresh garlic extracts(FGE) against *S. aureus* and *E. coli* with allicin, and clinically used antibiotics. The results of the study indicated that fresh garlic was more potent against *S. aureus* compared to allicin and not much difference in activity was noticed against *E. coli* while both bacteria were more sensitive to antibiotics than garlic extract or allicin (Fujisawa et al., 2009) which was Same with our results in which the control antibiotics of Augumentin was more active against all the resistant strains than the extracts.

In vitro antibacterial assays report that AGE is effective against various Gram-positive and Gram-negative oral bacteria, which include periodontal pathogenic bacteria Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and S. mutans (Bakri and Douglas, 2005; Fani et al., 2007; Velliyagounder et al., 2012). Different studies reported that AGE exhibited activity against a large variety of Gram-positive and Gram-positive pathogenic bacteria including MDR strains and isolates such as MDR M. tuberculosis showing not only the effectiveness of garlic against drug-resistant bacteria but also its broad spectrum (Iwalokun et al., 2004; Gupta et al., 2010; Gull et al., 2012; Meriga et al., 2012). In an interesting study, it was found that counts of S. aureus in hamburger upon addition of AGE reduced in a dose-dependent manner during storage for different time points in the fridge and freezer, supporting the idea of using garlic for meat preservation (Mozaffari Nejad et al., 2014). This was also in agreement with our study that showed where the extracts showed broad spectrum activity against the selected resistant strains of staphylococcus aureus and was also dose dependant mostly at an increased dosages.

To compare the antibacterial activity of various garlic health products, aqueous extracts of different products that included GP, GO, gelatinous GP suspension, aged garlic extract, and gelatinous suspension of aged garlic extract were prepared along with fresh garlic. All the extracts exhibited activity against Neisseria gonorrhoeae, S. aureus, and Enterococcus faecalis. The activity was correlated to the amount of fresh garlic constituents, namely, allicin and SAC, present in the products (Ruddock et al., 2005). The Burkholderia cepacia complex (Bcc) consists of 17 different species of soil bacteria that are pathogenic to allium species. These bacteria cause life-threatening lung infections in patients suffering from cystic fibrosis. AGE exhibited activity against Bcc, and this activity correlated with the allicin content of the extract (Wallock-Richards et al., 2014). In a recent study, non-aged and aged garlic cloves were pressed to remove their juices, dried, and powdered before extracted with water, ethanol, and chloroform. All three extracts of aged garlic exhibited antibacterial activity while only chloroform extract of non-aged garlic had activity against B. cereus (Jang et al., 2018). All these studies indicate that allicin is the main phytochemical responsible for the antibacterial activity of AGE. Although the ethanol extract of garlic also has allicin, AGE is more effective due to the presence of other

antibacterial chemicals, which might result in a synergistic or additive effect,same with our study that we suspect the presence of certain bioactive compounds like allicin in garlic in the bioactive activity against the resistant strains.

HPLC analysis of ethanolic extract of garlic (EGE) revealed that it contains various thoisulfinates, the major one being allicin. The anti-H. pylori activity of this extract decreased with the decrease in the concentration of allicin. Furthermore, it was seen that the maturation of garlic increases the allicin yield and extract with acetone yielding a higher percentage of allicin compared to ethanol (Canizares et al., 2004a, b). In vitro studies have reported that EGE was found to show antibacterial activity against various pathogenic bacteria including MDR bacteria, MDR M. tuberculosis isolates, and vancomycin-resistant S. aureus (VRSA) isolates (Hannan et al., 2011; Karuppiah and Rajaram, 2012; Snowden et al., 2014; Liaqat et al., 2015). The antibacterial and antiadherence activity of organic solvent (chloroform, acetone, and ethanol) extracts of garlic was least compared to crude and aqueous extract against S. mutans (Jain et al., 2015a) which agreed to our research where aqueous extract showed increased activity against the isolates compared to the ethanol.

CONCLUSION

Antimicrobial resistance has become a global phenomenon and posed a serious health challenge to the world. The development of resistance to antibiotics by most resistant bacteria strains like *staphylococcus aureus* and *Escherichia coli* could be largely attributed to misuse and overuse of antibiotics by clinicians, quacks, patients, farmers ,etc. this has reached to a zenith level of a possibility of causing crisis in the health sector, increase in cost of health care, increase of morbidity and mortality hence the need to intensify effort in research to screen for phytomedicine or alternative medicine with less side effect but huge potential activity and effect against drug resistant organisms. Fresh ethanol and aqueous garlic extracts from our study has shown sufficient evidence to be effective against drug resistance strains of *S.aureus* and *E.coli* hence the need for more research on the antibacterial potentials of various products of garlic against drug resistant organisms.

RECOMMENDATION

From the findings of this research we will recommend the use of various products of garlic extracts in combating the menance of drug resistance organisms and will also suggest further studies on its phytochemical contents, further antibacterial studies with dry or powered garlic extracts against several other multidrug resistant organisms.

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