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A REVIEW ON *IN VITRO* DETERMINATION OF BACTERICIDAL EFFECT OF GARLIC & GINGER ON STAPHYLOCOCCUS

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Abstarct: ***The actual aim of this review article is to get a thoroughly search on different in vitro studies on bactericidal effect of staphylococcus on various extracts of garlic and ginger. Microbial pathogenecity and other infectious diseases have been controlled by use of commercially accessible antimicrobial drugs since last many years. In recent years, in sight of their valuable effects, utilize of spices or herbs is gradually increasing not only in developing countries but also in developed countries. Garlic (Allium sativum) that belongs to the family of Alliaceae is widely used in culinary and medicine. In new researches, it is reported that garlic extract has been proved to be proficient toward Streptococcus mutans, garlic extract mouth wash may be utilized as a recent line in inhibiting dental caries formation. Garlic is highly documented in exhibiting dominant antimicrobial activities. Garlic can provide proper management for bacterial growth ranging from disinfectant, antiseptic, bacteriostatic and even bactericidal characteristics. Zingiber officinale plays role as powerful food maintenance. In limited studies, ginger was found to have better usefulness than placebo in relieving disgust produced by sea dizziness, morning dizziness, despite ginger is not reported to be preferred on placebo in relieving surgical sickness. The plant is reported to have antibacterial, anti-oxidant, antiprotozoal, anti-fungal, anti-emetic, anti-rhinoviral, anti-inflammatory, anti-insecticidal activity. Staphylococcus aureus is the most medically important member in terms of pathogenicity of the group, two other less important members are Staphylococcus epidermis and Staphylococcus saprophyticus.***

Keywords: Allium sativum, Zingiber officinale, microbial pathogenecity, Staphylococcus, in vitro studies.

INTRODUCTION:

Tremendous use of antibiotics has developed multiple drug resistance (MDR) in many bacterial pathogens. The increasing drug resistance is the main hindrance in successful treatment of infectious diseases and to the control of microbial pathogenecity [1]. Similarly, preservatives like sulfites, nitrates, nitrites and antibiotics, are harmful for human health and have many side effects including headache, nausea, weakness, mental retardation, seizures, cancer and anorexia [2]. Development of drug resistance in pathogens and increasing interest of consumers for safe food forces to explore new antimicrobial agents [3]. Natural products are a major source of new natural drugs and their use as an alternative medicine for treatment of various diseases has been increased in the last few decades [4,5]. In comparison to the formulated drugs the herbs and spices have fewer side effects. They are also inexpensive, show better patient tolerance and are readily available for low socioeconomic populatation [6].

In recent years, in view of their beneficial effects, use of spices or herbs is gradually increasing not only in developing countries but also in developed countries [7]. Microbial infections are the major cause of morbidity and mortality in the developed and developing country, although a number of antimicrobial agents are available for the treatment and management of infectious diseases [6]. Historically, garlic has been used for centuries worldwide by various societies to combat infectious disease. Garlic can be provided in the form of capsules and powders, as dietary supplements, and thus differ from conventional foods or food ingredients [8].

Garlic (*Allium sativum* L.) exhibit a broad antibiotic activity against both gram negative and gram positive bacteria including species of *Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus, Clostridium, Helicobacter pylori* [9-11]. One of the most potent and active component in garlic is the Sulphur compound called allicin which is a chemical compound produced when garlic is chopped, chewed or bruised. It is a powerful antibiotic and an agent that helps the body to inhibit the growth and development of pathogenic microbes [12].

Garlic (*Allium sativum*) that belongs to the family of *Alliaceae* is widely used in culinary and medicine [13]. Aqueous, methanol and ethanol extracts of *A. sativum* has been reported to possess antimicrobial effect against drug resistant organisms such as *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, S. aureus, Klebsiella pneumoniae, Shigella sonnei, Staphylococcus epidermidis* and *Salmonella typhi* [14-16]. *A. sativum* is primarily a bacteriostatic agent that destroys sulfhydryl groups necessary for bacterial growth [17].

Zingiber officinale plays role as powerful food maintenance. In limited studies, ginger was found to has better effectiveness than placebo in relieving disgust produced by sea dizziness, morning dizziness, despite ginger is not reported to be preferred on placebo in relieving surgical sickness. The plant is reported to have antibacterial, anti-oxidant, antiprotozoal, anti-fungal, anti-emetic, anti-rhinoviral, anti-inflammatory, anti-insecticidal activity. Reported pharmacological activities of ginger include antipyretic, analgesic, ant tissues in addition to hypersensitive effects [18 & 19].

Table 1: A brief summary of plants

Plant	Family	Medicinal use	Reference
Garlic (*Alium sativum*)	Amaryllidaceae	It has antiseptic, anticoagulant, antibiotic, antioxidant, analgesic, anti-inflammatory and anti-cancerous properties.	[20-22]
Ginger (*Zingiber officinale*)	Zingiberaceae	It has antimicrobial, antioxidant, anti-cancerous, anti-inflammatory.	[23-26]

Table 3: Phytochemical screening of plants extracts [20-26]

Phytochemicals	Herbal Plants	
	Garlic	Ginger
Phenols	+	+
Flavonoids	+	+
Saponins	+	+
Steroids	+	-

Tanins	-	+
Glycosides	+	+
Terpenoids	+	+
Alkaloids	+	+
Carbohydrates	+	+

S. aureus, a common cause of human infections have been recognized as pathogen of high public health significance. *S. aureus* is a normal inhabitant of the human skin and the respiratory tract; and, nasal presence of *S. aureus* plays a key role in the development of *S. aureus* infections [27-28].

GARLIC

Garlic is the common name of the genus *Allium sativum*. *Allium sativum* can be considered as a national product of many centralized Asian countries. Moreover, garlic had been utilizes for longer period of time in the areas of Mediterranean, likewise a condiment in more than one continent including Europe Africa, as such Asia. Garlic had been well identified for antique Egyptians, as well as utilized in cooking and in remedial goals. *Allium sativum* follows to the family Alliaceae. Plants of *Allium sativum* can be developed in a close proximity to each other, giving up proper area to enhance the maturation of the bulbs, and are simply developed in vessels of appropriate deepness. Garlic does well in loose, dry; well drained soils in sunny locations. There are various kinds or subdivisions of *Allium sativum*, more obviously softneck and hardneck garlic. Hardneck garlic has been usually developed in neerly cool wheather while softneck garlic is usually well developed in close vicinity to the equability line. There are several works had been made on laboratory animals and human being that proves the beneficial cardiovascular property of (garlic). When both ginger and garlic used as a natural supplement, this considered as a healthier choice for many diseases (Alzheimer's disease as example). *Allium sativum* was utilized to inhibit gangrene formation through the first and Second World War. In new researches, it is reported that garlic extract has been proved to be efficient toward *Streptococcus mutans*, garlic extract mouth wash may be utilized as a modern line in inhibiting dental caries formation. Garlic is highly documented in exhibiting powerful antimicrobial activities. Garlic can provide proper management for bacterial growth ranging from disinfectant, antiseptic, bacteriostatic and even bactericidal characteristics. Besides that, garlic may have the ability to prevent and manage viral, fungal and even helminthes infections. Newly obtained garlic has been found to impart a significant role in managing food poisoning through killing the causative agents such as Escherichia coli. Recently, it has approved that there is a possibility of using garlic in preserving meats from bacterial spoilage and this related to the antibacterial activity of garlic. Upon treatment of meat with garlic, bacterial numbers were significantly diminished in comparison with the non-treated meat when both meats were kept in refrigerator at 4°C [29-33].

Allicin is an organosulfur compound found in garlic (the active ingredient), it shows inhibitory effect on some pathogenic bacteria. The best known and well studied effect of Allicin was illustrated by controlling and killing activity to *Staphylococcus aureus* (MRSA). *Allium sativum* could manage and regulate the oxidative stress status by trapping (binding and subsequent deactivating) the harmful oxidant agents (free radicals) [34-35].

Garlic (*Allium sativum*) has had an important dietary and medicinal role for centuries. It is a large annual plant of the Liliaceae family, which grows in most of Africa and in Ethiopia. Ethiopian garlic is used in traditional medicine for infectious disease and some other cases. The present study tested the aqueous extract of garlic *in vitro* for its antibacterial activity. The extract showed concentration dependent antibacterial activity against *Staphylococcus aureus*. The traditional use of Ethiopian garlic for infectious diseases and for controlling fever appears to be justified [36].

Allium sativum is a scientific name. it is commonly known as garlic is an odoriferous plant belonging to a *Lilacease* family. Garlic is a common plant and easily provides the market. It is a small perennial herb with narrow flat leaves and is confined all sides by membranous patches, garlic grown mostly in Northern Nigeria. The medicinal property of garlic due to its "Sulphur" content which was believed to be responsible for it is medicinal value. Raw garlic is used to treat colds and coughs. Garlic is an herbal ingredient for lowering high blood pressure Fighting heart alignments and cholesterol. It is used mainly for spice and also for its medicinal property. Garlic is our good because Garlic is rich in compounds like Allicin, Sulphur, Zinc, and Calcium that have health benefits, beauty benefits as well as antibiotics and antifungal properties. It is also a rich source of selenium. Selenium is known to fight cancer and it works with vitamin E in the body to boost antioxidant powder. Garlic as a medicinal plant has been widely used and found to be very effective of infections Other plants like the ones mentioned earlier also have their medicinal properly, some which is as a result of the presence of alkaloids, volatile oils, polyphenol and some related Sulphur compound contained in them. Similarly, some are found to be used as vermifuge, stimulating carminative toxic and also as condiments and also as condiments and for treatment of worm bites just like garlic. Garlic is a mostly useful for medicinal, and control to infections. Garlic has been used from the time when ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease and regular use of garlic may help to prevent cancer, to treat malaria, and to raise immunity. Garlic has also proposed to treat asthma, candidiasis, colds, diabetes, and antibacterial effect against food borne pathogens like *S. aureus [37]*.

Garlic as an antibiotic

Garlic is an anti -bacterial agent that can actually inhibits growth of infectious agents and at the same time protect the body from the pathogens. It is known that the most sensitive bacterium to garlic is the deadly *Bacillus anthracis* which causes the diseases anthrax. Even the forefather of antibiotic medicine Louis Pasture acknowledged garlic to be an effective antibiotic. Some year's later garlic was shown to have similar effect/activity as penicillin. Later studies should similar activity to modern antibiotic including Chloramphenicol. Even the blood of garlic eaters can kill bacteria and it is also reported that the vapor from freshly cut garlic can kill bacteria at a distance of 20 cm! The other, the common and apparently returning diseases tuberculosis was treated with garlic very successfully as invading *Mycobacterium tuberculosis* is sensitive to several of the sulphur components found in Garlic [38,39].

Allicin

Allicin is the antibacterial component found in Garlic. A molecular mechanism may be the basis for some of garlic's therapeutic effects. The researchers were able to study how garlic works at molecular level using allicin, garlic's main biologically active component. Allicin created when garlic cloves are crushed, protects the plant from soil parasites and fungi and is also responsible

for garlic's pungent smell. It is a natural weapon against infection that disables dysentery causing amoebiasis by blocking two groups of enzymes, cysteine proteinases and alcohol dehydrogenases. Cysteine proteinases enzymes are the main culprits in infection, providing infectious organisms with the means to damage and invade tissues. Alcohol dehydrogenase enzymes play a major role in these harmful organisms' survival and metabolism. Because of these groups of enzymes are found in a wide variety of infectious organisms such as bacteria, fungi and viruses. This research provides scientific bases for the notion that allicin is a broad-spectrum antimicrobial, capable of warding off different types of infections. It is likely that bacteria would develop resistance to allicin because this would require modifying the very enzymes that make their activity possible scientists found that allicin blocks the enzymes by reacting with one of their important components known as self sulfadryl (SH) groups, or thiols this finding has important implication because of sulfadryl groups are also crucial component of some enzymes that participate in the synthesis of cholesterol "Garlic lowers the level of harmful cholesterol'' [38-41].

GINGER

Ginger (ginger rhizome) is the root of the *Zingiber officinale* plant, which can be utilized as a medication or as pleasant condiments. Ginger derived its name from the genus (*Zingiber officinale)* and the family (Zingiberaceae). In addition to ginger, there are other important followers to this plant family including turmeric, cardamom and galangal1. *Zingiber officinale* creates sets of flower sprouts (pink and white) that developed into yellow flowers. As a result of the beautiful appearance and the habituation of plant to hot weather, *Zingiber officinale* is usually utilized as scenery across subequatorial homes. Fully developed *Zingiber officinale* roots are fibrous and approximately arid. The syrup from ancient ginger rhizomes is highly strong and usually utilized like condiments in Indian prescription, and is as typical component of cooking in many Asian countries also this return to its pleasant relish which makes the taste of many food dishes an extremely delicious [42,43].

Ginger (*Zingiber officinale*) is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases. Ginger has direct antimicrobial activity and thus can be used in treatment of bacterial infections. Ginger belongs to Zingiberaceae family. [44-54] The Zingiberaceous plants have strong aromatic and medicinal properties and are characterized by their tuberous or non tuberous rhizomes. Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. In many countries including Bangledesh, ginger is used in boiled food preparation. *Staphylococcus aureus* is the most medically important member in terms of pathogenicity of the group. Two other less important members are *Staphylococcus epidermis and Staphylococcus saprophyticus*. It is commonly present in the upper respiratory tract and intestinal tract. They grow on a variety of media and ferment many carbohydrates without gas. Colonies may be white, grey or golden yellow. The species name aureus is derived from the Latin word, 'aureus' meaning golden. They are halophilic (salt loving) [55-60].

LITERATURE SURVEY:

Ponmurugan Karuppiah, Shyamkumar Rajaram,, 2012, [61], evaluated on the antibacterial properties of *Allium sativum* (garlic) cloves and *Zingiber officinale* (ginger) rhizomes against multi-drug resistant clinical pathogens causing nosocomial infection. The cloves of garlic and rhizomes of ginger were extracted with 95% (v/v) ethanol. The ethanolic extracts were subjected to antibacterial sensitivity test against clinical pathogens. Anti-bacterial potentials of the extracts of two crude garlic cloves and ginger rhizomes were tested against five gram negative and two gram positive multi-drug resistant bacteria isolates. All the bacterial isolates were susceptible to crude extracts of both plants extracts. Except *Enterobacter sp*. and *Klebsiella sp.*, all other isolates were susceptible when subjected to ethanolic extracts of garlic and ginger. The highest inhibition zone was observed with garlic (19.45 mm) against Pseudomonas aeruginosa (*P. aeruginosa*). The minimal inhibitory concentration was as low as 67.00 稫g/mL against *P. aeruginosa*. Natural spices of garlic and ginger possess effective anti-bacterial activity against multi-drug clinical pathogens and can be used for prevention of drug resistant microbial diseases and further evaluation is necessary.

Mohamed A. Eltaweel,, 2014, [62], was extracted with the methanol and aqueous suspensions of the dried *Allium sativum* (Liliaceae) bulbs and it was screened for its anti-microbial activity using the agar-well diffusion method. It is tested against Gram-positive bacteria (*Staphylococcus aureus*). The suspensions were tested at concentrations of 1, 10, 100 and 1000 μg/ml. All suspensions showed an inhibitory effect against tested bacteria. The highest zone of inhibition was estimated with the highest concentration of aqueous suspension (48 mm) followed by the highest concentration of the methanolic suspensions (1000 μg/ml) which reached to 34 mm. The other concentrations either methanolic or aqueous showed various inhibitory effects on the tested bacteria.

Fariba Najafi, et *al.*, 2016, [63], was assessed antibacterial effects of *A. sativum* against *Staphylococcus aureus* (*S. aureus*) in west of Iran (in Kermanshah). Increasing bacterial resistance to chemical antibiotics and their probabilistic side effects cause popularity of medicinal plants, so there is an instantaneous and steady need for novel antibacterial compounds from plants. As we know, there is no documented proof on antibacterial activities of *Allium sativum* (*A. sativum*) essential oil in west of Iran. The antibacterial effects of *A. sativum* essential oil were evaluated by macro-dilution method in Mueller-Hinton broth medium, agar disk and well diffusion methods. The results indicated that the essential oil of *A. sativum* have inhibited the growth of *S. aureus* and destroyed it. Also, by increasing the concentration of the *A. sativum* essential oil, the inhibition zones increased. We believe that the article provide support to the antibacterial effects of the essential oil. Our finding shows the fact that the essential oil of *A. sativum* can be useful as medicinal or preservatives composition.

Manu Yadav *et al.* , 2019, [64], was conducted to test anti -bacterial activity of garlic against *Staphylococcus aureus* and *Escherichia coil.* The antibacterial effects of Aqueous Garlic Extract (AGE) against gram-positive and gram-negative bacterial isolates, *Staphylococcus aureus* and *Escherichia coli* were studied. Antibacterial activity of different concentrations of Aqueous Garlic Extract (AGE) by Well- Diffusion Method. Garlic extract was used in the range of100 % to 5 % (1ml/mL, 0.5 ml/ ml, 0.25 ml/ml, 0.125 ml/ ml and 0.625 ml/mL), against *Staphylococcus aureus* and *Escherichia coli* respectively. Further analysis revealed the antimicrobial efficacy of Aqueous Garlic Extract (AGE) is time and temperature dependent. These results suggest that

garlic have anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli* and can be used against pathogenic microorganisms.
Yalemwork Ewnetu *et al.*, 2014, [65], evaluated antimicrobial effects of mixtures of Ethiopian honeys and ginger rhizome powder extracts on *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (MRSA), *Escherichia coli* (R), and *Klebsiella pneumoniae* (R). Agar diffusion and broth assays were performed to determine susceptibility of these standard and resistant clinical bacteria isolates using honey-ginger powder extract mixtures. Honey-ginger powder extract mixtures produced the highest mean inhibition (25.62mm ± 2.55) compared to the use of honeys (21.63mm ± 3.30) or ginger extracts (19.23mm± 3.42) individually.The ranges of inhibitions produced by honey-ginger extract mixtures on susceptible test organisms (26–30 mm) and resistant strains (range: 19–27 mm) were higher compared to 7–22mm and 0–14mm by standard antibiotic discs. Minimum inhibitory concentrations (MIC) of mixture of honeys-ginger extracts were 6.25% (0.625 v/mL) on the susceptible bacteria compared to 75% for resistant clinical isolates. Minimum bactericidal concentration (MBC) of honey-ginger extracts was 12.5% (0.125 g/mL) for all the test organisms. *Conclusion*.The result of this study showed that honey-ginger powder extract mixtures have the potential to serve as cheap source of antibacterial agents especially for the drug resistant bacteria strains.

Bandna Chand, 2013, [66], was evaluated on the antibacterial activity of extracts of *Allium sativum* (garlic) and *Zingiber officinale* (ginger) against four different bacteria namely *Escherichia coli*, *Salmonella* Typhi, *Staphylococcus aureus* and *Bacillus cereus.* Two methods were used to determine the antimicrobial activity of garlic and ginger extracts namely disk diffusion method and agar well diffusion method. Garlic extract exhibited excellent antibacterial activity against all four test organisms while ginger extract showed antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* only. In addition, agar well diffusion method showed higher zone in inhibition when compared with the zone of inhibition produced by the spice of same concentration against the test microorganism by disk diffusion method. Antibiotic sensitivity of the four different bacteria was tested with commercially available antibiotics namely Ciprofloxacin; Oxytetracycline; Vancomycin; Streptomycin; Gentamicin; Tetracycline; Novobiocin; Amikacin and Penicillin G. Penicillin G produced the highest zone of inhibition of 40.00±0.00against *Staphylococcus aureus* and the lowest zone of inhibition of 0.00±0.00 against *Escherichia coli.*

Wolde T, *et al.*, 2018, [67], was conducted to evaluate the anti-bacterial effect of garlic against standard isolates of *S. aureus and E. coli* kindly obtained from EHNRI. In many developing countries a large proportion of the population relies on traditional practitioner of medicinal plants in order to meet health care need. Garlic is one of the herbs that used by traditional practitioners for preparation of herbals medicine. Emergence of drug resistance is obvious and global confront. Seeking for other antibiotics which are new, natural, plant based. Garlic is classified as member of family Alliaceae. Allicin is one of the active principal of freshly crushed garlic homogenates, have variety of antimicrobial activities. Four different solvents having different polarity were used to extract the bioactive compound from garlic. The Antibacterial activity of the crude extracts of garlic was evaluated against Standard isolates of *S. aureus and E. coli* by an agar diffusion method. The trial was done in triplicates. A Factorial Design with three factors was used. The treatment means were compared by a Student's t- test with least significant

difference (LSD) at 5% (P=0.05) and the data analysis was performed using mini tab statistical software package. In this experiment the non-polar chloroform had higher inhibition zone. The highest yield potential was obtained from water followed by ethanol, chloroform and petroleum ether respectively. *E. coli* were so susceptible than *S. aureus* to the extracts. Garlic could be used as effective antibacterial agent for human pathogenic bacteria.

Abiy E, Berhe A., 2016, [68], was conducted to evaluate the anti-bacterial effect of garlic against clinical and standard isolates of *S. aureus and E. coli* from patients attending Hawassa University. Emergence of methicillin drug resistance is evident and global challenge. Seeking for alternative antibiotics which are new, natural, plant based, cost effective and in toxic is the up to date task for global health. The Antibacterial activity of the crude extract of garlic was investigated against Clinical and Standard isolates of *S. aureus and E. coli* by an Agar of both dilution and Cork borer techniques. The trial was done in triplicates. The results showed that standard *S. aureus and E. coli* were completely inhibited by 10 mg/ml and 15 mg/ml of agar media respectively and their clinical isolates were completely inhibited by 25 mg/ml, indicating that standard isolates are most sensitive and clinical isolates are least sensitive. Garlic could be used as effective antibacterial agent for these pathogenic microorganisms.

Augustine I. Airaodion1 *et al.*, 2020, [69], was determined the antibacterial activity of the juice by diffusion method. Emergence of methicillin drug resistance is evident and has become a global challenge. Seeking for alternative antibiotics that are new, natural, plant based, cost effective and less toxic is the recent task for global health. This study is aimed at assessing the pharmacotherapeutic activity of *Allium sativum* (Garlic) bulb against gram-positive and gram-negative bacteria. Fresh *A. sativum* bulbs were purchased from a local market in Ibadan, Nigeria and were identified by a botanist. They were cut into small pieces and mashed in a laboratory with a mortar and pestle and the fluid squeezed out of the resultant slurry. Nutrient agar medium was prepared using standard method. Pure cultures of *Coliform bacillus, Staphylococcus epidermidis, Streptococcus viridians, Salmonella typhi and Escherichia coli* were obtained from the Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture, Abeokuta, Nigeria. The juices were serially diluted to obtain 1.0%, 0.5%, 0.25% and 0.125% solutions in sterile test tubes. Sterilized 9 mm filter paper disc soaked in the diluted juice were placed on the plate and incubated for 24 hours at room temperature. The plates were examined for clear zones of inhibition. Presence of zones of inhibition indicated activity. The results showed that *A. sativum* bulb has antibacterial potential against all the bacteria used in this study and also exhibited inhibitory activity against them. The result of this present study showed that *A. sativum* juice has high range of antibacterial potential against both gram positive (*S. epidemidis and S. viridans*) and gram negative bacteria (*C. bacillus, E. coli and S. typhi*). However, the extract has a greater inhibitory activity against gram positive bacteria than gram negative bacteria.

Bulti Kumera Fufa, 2019, [70], reported that garlic (*Allium sativum*) contains various biologically active components that play a significant role in the treatment of bacterial and fungal infections. It contains sulfur compounds like allicin, ajoene, allyl methyltri sulfide, diallyl trisulfide, di allyl di sulphide and others which exhibit various biological properties like antimicrobial, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and cardiovascular effects. The objective of the current review was to relate various literatures

and assess the anti-microbial potential of garlic extract. The antimicrobial potency of garlic can be maximized by increasing the concentration of the extract. Garlic extract of 100% concentration showed a maximum zone of inhibition against both gram-positive and gram-negative bacteria.

Ashok kumar *et al.*, 2018, [71], were used for relative analysis of antioxidant and antimicrobial activity on ethanolic extracts of Garlic (Bulb), Aloe (leaf), Flower bud (buds), Turmeric (rhizomes) and Ginger (rhizomes). Antioxidant activity was determined by DPPH [1, 1-Diphenyl-2-picryl hydrazyl] assay and expressed with Ascorbic acid. It was observed that turmeric and ginger have more antioxidant activity than garlic, Aloe and Flower bud. These extracts were further studied for antibacterial activity by agar well diffusion and spectrophotometric method against tetracycline as reference. The result showed that Flower bud is more effective against *Escherichia coli, Pseudomonas aeuroginosa, Bacillus subtilis* and *Staphylococcus aureus* compared to other plants extract. However, all the plants extract did show antioxidant and antibacterial activity.

Ashraf A. Mostafa *et al.*, 2018, [72], reported that prevention of food spoilage and food poisoning pathogens is usually achieved by use of chemical preservatives which have negative impacts including: human health hazards of the chemical applications, chemical residues in food & feed chains and acquisition of microbial resistance to the used chemicals. Because of such concerns, the necessity to find potentially effective, healthy safer and natural alternative preservatives is increased. Within these texts, Plant extracts have been used to control food poisoning diseases and preserve foodstuff. Antimicrobial activity of five plant extracts were investigated against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi* using agar disc diffusion technique. Ethanolic extracts of *Punica granatum, Syzygium aromaticum, Zingiber officinales and Thymus vulgaris* were potentially effective with variable efficiency against the tested bacterial strains at concentration of 10 mg/ml while extract of Cuminum cyminum was only effective against *S. aureus respectively. P. granatum and S. aromaticum* ethanolic extracts were the most effective plant extracts and showed bacteriostatic and bactericidal activities against the highly susceptible strains of food borne pathogenic bacteria (*S. aureus and P. aeruginosa*) with MIC's ranged from 2.5 to 5.0 mg/ml and MBC of 5.0 and 10 mg/ml except *P. aeruginosa* which was less sensitive and its MBC reached to 12.5 mg/ml of *S. aromaticum* respectively. These plant extracts which proved to be potentially effective can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding healthy hazards of chemically antimicrobial agent applications.

Sapkota *et al.*, 2012, [73], studied the antibacterial effect of guava leaves, garlic and ginger against some human microbial pathogens and they ascertained that ginger was only effective against *S. aureus* while guava and garlic were effective against all tested microorganisms.

Qader *et al.*, 2013, [74], were investigated on seven ethanolic and aqueous plant extracts against some clinically pathogenic bacteria. Ethanolic *Punica granatum* extract was effective against all tested bacterial pathogens with MIC of 0.2 mg/ml. *Zingiber officinales* extract was also effective against *P. aeruginosa* and *K. pneumonia while Thymus kotschyana* was potentially effective against *S. aureus and E. coli*.

Iram Gull *et al.*, 2012, [75], reported that herbs and spices are very important and useful as therapeutic agent against many pathological infections. Increasing multidrug resistance of pathogens forces to find alternative compounds for treatment of infectious diseases. In the present study the antimicrobial potency of garlic and ginger has been investigated against eight local clinical bacterial isolates. Three types of extracts of each garlic and ginger including aqueous extract, methanol extract and ethanol extract had been assayed separately against drug resistant *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Shigella sonnei, Staphylococcus epidermidis and Salmonella typhi*. The antibacterial activity was determined by disc diffusion method. All tested bacterial strains were most susceptible to the garlic aqueous extract and showed poor susceptibility to the ginger aqueous extract. The (minimum inhibitory concentration) MIC of different bacterial species varied from 0.05 mg/ml to 1.0 mg/ml. In the light of several socioeconomic factors of Pakistan mainly poverty and poor hygienic condition, present study encourages the use of spices as alternative or supplementary medicine to reduce the burden of high cost, side effects and progressively increasing drug resistance of pathogens.

V. U. Olugbue *et al.*, 2017, [76], assessed on the antibacterial potentials of *Allium sativum* and their interaction with antibiotics against clinical isolates of multidrug resistant Staphylococcus aureus. Study Design: The study is designed to investigate the antibacterial activity of *Allium sativum* extract and its interactive potential to enhance the activity of antibiotics against multidrug resistant *S. aureus*. The study was conducted between May, 2015 and June, 2016. This study was performed at the Microbiology laboratory of the Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria. Extraction was done using sterile water, acetone and methanol. The extracts were evaporated to dryness using rotary evaporator. Antibacterial susceptibility testing was carried out using the agar well diffusion method. The macrobroth dilution method was used to determine the MIC of the methanol extracts. Checkerboard method was used to determine the interaction between extracts and antibiotics. Aqueous and methanol extracts of *Allium sativum* had inhibition zone range of 10 ± 1.50 to 17 ± 1.50 mm and 7 ± 1.05 to 14 ± 0.95 mm respectively, at concentration range of 25 to 200 mg/ml. Acetone extract had inhibition range of 5 ± 0.50 to 11 ± 0.50 mm. Methanol and acetone extracts had the same inhibition zone (14 ± 0.79 mm) diameter against the *S. aureus* isolates at 200 mg/ml. There were no significant difference found between inhibition zone diameter of methanol/acetone extract ($P = 0.50$), methanol/aqueous extracts ($P = 0.97$) and acetone/aqueous extracts ($P = 0.48$) against the test bacteria. The correlation analysis done between inhibition zone diameter and concentration of *A. sativum* recorded a positive correlation (r) ranging between 0.84 and 0.89. The combination of methanol extracts of garlic (MEG) plus ceftazidime (Caz) and MEG plus erythromycin (Ery) showed synergistic interaction in three and four of the isolates respectively. Antagonistic interaction was recorded in the combination of MEG plus cefuroxime in one isolate whereas it was recorded in three isolates in the interaction of MEG and ciprofloxacin. The synergistic interaction stood at 28.0% and antagonism 16.0%. The combination of ½ x MIC of MEG plus ½ x MIC Caz against *S. aureus* showed bactericidal activity against the isolates at 12 hours resulting in a 4.25 log10 cfu/ml reduction whereas the combinations of ½ x MIC of MEG plus ½ x MIC of they showed bactericidal activity after 8 hours with a 3.13 log10 cfu/ml decrease. The net reduction in colony counts was observed consistently between 12 – 24 hours. The result of this study showed that methanol extract of *A. sativum* possesses bactericidal activities against *S.*

aureus. In addition, the methanol extract may be a potential source of resistance modifying compounds that can potentially improve the performance of antibiotics in the treatment of multidrug resistant *S. aureus* infections. There is need to isolate the specific active agent(s) involved in the potentiation for drug compounding.

Okiemute Patricia Anyamaobi *et al.*, 2020, [45], were investigated on the antimicrobial properties of garlic (*Allium sativum*) and ginger (*Zingiber officinale)* for their microbial activity against both clinical and laboratory isolates of Staphylococcus aureus using disc diffusion method. Ten isolates were obtained from the Department of Medical Microbiology, Medical Laboratory science Madonna University Teaching Hospital, Elele. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the broth dilution method. The antibiotic susceptibility test against the test organisms were performed by disc diffusion method. The result from this study showed garlic and ginger extract were effective against *Staphylococcus aureus* used in this study. The inhibitory effect exerted by garlic extract was highest at the concentration of 100µg/ml with mean zone inhibition of 11.5 ± 0.69mm which was statistically significant ($p<0.05$) from the control 21.6 ± 1.07mm. Also the inhibitory effect exerted by garlic was least at the concentration of 12.5µg/ml with mean zone of inhibition of 3.5 ± 0.46mm, while concentration at 50µg/ml showed mean zone of inhibition of 8.9 ± 1.11mm and concentration at 25µg/ml showed mean zone of inhibition of 5.6 ± 0.55mm. The inhibitory effect exerted by ginger was highest at the concentration of 100µg/ml with mean zone inhibition of 16.0 ± 0.21mm which was statistically significant ($p<0.05$) from the control 21.6 ± 1.07mm. Also the inhibitory effect exerted by ginger was least at the concentration of 12.5µg/ml with mean zone of inhibition of 4.5 ± 0.68mm, while concentration at 50µg/ml showed mean zone of inhibition of 11.7 ± 1.05mm and concentration at 25µg/ml showed mean zone of inhibition of 8.0 ± 0.89mm. *Staphylococcus aureus* was found to be more sensitive to ginger than garlic with highest mean zone inhibition of 16.0 ± 0.21mm for ginger and 11.5 ± 0.69mm for garlic at 100µg/ml. Also there were lower minimum inhibitory concentrations with 25µg/ml for garlic and 12.5µg/ml for ginger on *Staphylococcus aureus* than the minimum bactericidal concentrations with 50µg/ml for garlic and 25µg/ml for ginger indicating that the extracts could be bactericidal in action. Therefore garlic and ginger extracts can be used as a source of antibiotic substances for possible treatment of staphylococcal infection though not replacing the use of antibiotics but to prevent multidrug resistance.

METHODOLOGY:

Aliumsativum (garlic) and Zingiberofficinale (ginger) Sensitivity Testing of Bacterial Strain [77]:

Pure culture of *Staphylococcus aureus* was subjected to antimicrobial susceptibility using the disc diffusion method. Using a sterile wire loop, pure colonies of the test organism was aseptically inoculated in 3mls of sterile normal saline and compared with McFarland standard and was allowed to incubate for 5minutes. A sterile swab stick was dipped into the bacterial suspension; excess inoculum was reduced by pressing the swab stick against the wall of the test tube. Inoculum was spread on the surface of Mueller Hinton agar and was allowed to stay for 5 minutes to dry. A sterile forcep was used to pick the impregnated extract disc unto Mueller Hinton agar and was incubated at 37^0 c for 24hrs. The area of inhibition was examined and measured in milliliters. *Serial dilution of Aliumsativum (garlic) and Zingiberofficinale (ginger)*

Serial tube dilution technique was used to determine the minimum inhibitory concentration (M.I.C.). The garlic cloves and ginger rhizomes crude extracts were serial diluted in the range from 12.5 to 100µg/ml. The tubes were inoculated with 100µl of bacterial culture at a concentration of 10 6cells/ml. Standard antibiotic ciprofloxacin was included in the assay for comparison. Peptone water with the inoculum only was used as a growth control. All experiments were carried out in triplicates. The tubes were incubated aerobically at 37˚C for 18hours.

Determination of Minimum Inhibitory Concentration (M.I.C) and Minimum Bactericidal Concentration (M.B.C) [67]:

Antimicrobial agents were diluted in peptone water. Each dilution was inoculated with standard inoculum of test organism. After appropriate incubation, the lowest concentration in the tube that showed visible inhibition of growth was recorded as the minimum inhibitory concentration (M.I.C). Dilution with test organism were then subcultured on blood agar medium without antimicrobial agent and incubated for 24hrs at 37^0 C. The minimum bactericidal concentration (M.B.C) is the lowest concentration of antimicrobial agent that produces no bacterial growth on a solid medium. M.I.C and M.B.C were expressed as microgram / milliliters..

Antimicrobial susceptibility test by Kirby-Bauer method [77]:

The antibiotics susceptibility procedure for bacterial spices has been done through using a method that depends on the ability of disc to permit the penetration of antibiotics through the medium which is also called Kirby-Bauer method 15. The overall steps of the procedure should be produced through entirely sterilized status. Plates of Mueller Hinton agar have been inoculated; each alone, by the test bacterial isolates -107 CFU (compared with Mcfarland turbidity standard), then the bacterial suspension was uniformly distributed overall the area of the plate. After a while, sterilized discs (measuring five milliliters in diameter) were put under sterilized conditions in various extracts (for about 1 min), then fixed on Mueller Hinton plates (petridishes) inoculated previously by suspension of the bacteria. After this step, all plates had been put aside (at 250C for about 15 mins). After that, all cultured plates were placed in the incubator at 36°C for 18-20 hrs; the area of inhibition has been examined and calculated in millimeters (mm).

The organisms under the experiment were evaluated, as well, for their susceptibility toward some antimicrobials including: cloxacillin, cefepime, cefoxitin, clindamycin and tobramycin, again by disc diffusion procedure. The cultures of test organisms were reactivated by culturing in sterile nutrient broth for 16 hrs at 37°C. After incubation and turbidity comparison with Mcfarland turbidity standard, sterile cotton swabs were used to transfer the bacterial cultures aseptically and swabbed over Mueller Hinton agar petridishes. A sterilized forceps was used to fix the antibiotic disc aseptically over the cultured petridishes. Then, the petridishes were placed in the incubator at 37°C for 20-22 hours and subsequently all diameters of inhibition zones could be determined.

Reported Results [77]

The inhibition zones that obtained by the garlic and ginger extracts toward the bacterial isolates were measured as a determination of inhibitory effects of these plants. All results obtained above in table-2 were resulted by disk diffusion method (Kirby-Bayer). In addition to that, ethanolic disks were used to all test isolates (as a control) and all these disks with no value (no zones of inhibition have been occurred).

The results in table-2 revealed that the aqueous extract of ginger and garlic (each alone) gave no effect (against Streptococcus pyogens and Pseudomonas aeruginosa,) and low level of inhibition

on other isolates. Moreover, the combination of aqueous extract of them gave slightly higher level of inhibition than that of each extract alone. On the other hand, the ethanolic extract of ginger and garlic (each alone) showed variable level of inhibition (ranging from 9mm to 20mm) and this indicated that the ethanolic extraction gives better extraction than that of aqueous one. Additionally, the combination of ethanolic extract of both ginger and garlic produced broader zone of inhibition than that of each extract alone, this mean that there is a potentiation-like effect between both extract. The important thing is that Streptococcus pyogens was completely resistant to all extracts.

Muhsin Dalia Abdulzahra and Hussein Furqan Mohammed (2014 [77],), Newly obtained rhizomes of *Zingiber officinale (Ginger) and Cloves of Allium sativum* (Garlic) were put together, leaved nearly at 250C to permit air-drying, milled to fine powder and then these powders would be extracted (each alone) using water and ethanol as solvents for the extraction. After that, the extracts were examined for its antibacterial (inhibitory) effect toward some clinical isolates (patient with otitis media) of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogens (G+ve) and Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis* (G-ve). In this study, the antibacterial (inhibitory) effect of the extracts of both ginger and garlic has been determined toward six clinical bacterial isolates (mentioned previously). Two kinds of extracts for ginger and two kinds of extracts for garlic have been obtained (involving watery extract and ethanolic extract) and then examined separately and in combination of these extract. In the present study, some antibiotics (cloxacillin, cefepime, cefoxitin, clindamycin and tobramycin) were used to compare their effect with the effect of the extracts obtained. Disc diffusion method (Kirby-Bauer method) was used to determine the antibacterial activity of extracts. The test isolates showed variable susceptibility to the garlic and ginger extract (aqueous and ethanolic) and to other antibiotics (cloxacillin, cefepime, cefoxitin, clindamycin and tobramycin). The outcomes of susceptibility experiment depicted that ethanolic extract of garlic and ginger (each alone and in combination) showed more inhibitory effect than aqueous extract and also the combination of ethanolic extract of both ginger and garlic resulted in inhibitory effect greater than each extract alone. Both ginger and garlic extract have antibacterial activity (especially the ethanolic extract) against some pathogenic G+ve and G-ve bacteria.

Antimicrobial assay using Disc diffusion method

The antimicrobial assay of spices was performed by disc diffusion method as described by Kirby-Bauer [78]. All the experiments were performed under sterile conditions. The nutrient agar plates were inoculated separately with 107 CFU of each test bacterial strain culture and evenly spread on entire surface of each plate. The sterile discs (5 mm diameter) were dipped aseptically in different extracts for one minute and placed over nutrient agar plates seeded with bacterial culture. The plates were left at ambient temperature for 15 minutes and then incubated at 37°C for 16 hours and observed for zone of inhibition. The diameter of inhibition zones was measured in millimeters. Antimicrobial assay was performed in triplicate with each bacterial strain.

Determination of minimum inhibitory concentration (MIC)

MIC of different garlic and ginger extracts was determined by the method described by Natta et al [79] after minor modifications. The extracts were diluted ranging from 100 mg/ml to 0.01 mg/ml and checked for MIC against bacterial strains. Sterile discs were dipped in different

dilutions of aqueous, ethanol and methanol extracts of garlic and ginger and placed over LB agar plates seeded with 107 CFU of each bacterial culture separately. Plates were placed at 37°C for 16 hours. The zone of inhibition in each case was measured as the diameter of the clearing zones and results were recorded. Each experiment was performed in triplicate.

Antibacterial Susceptibility Testing of Plant Extract [80-82]

The antibacterial susceptibility testing of *A. sativum* extracts against isolates of *S. aureus* was carried out using the agar well diffusion method. A sterile Pasteur pipette was used to drop 0.2 ml standardized inoculums equivalent to 0.5 McFarland turbidity standards on the surface of already prepared and dry Mueller- Hinton agar. The inoculum was evenly spread using Hockey stick shaped glass rod. Six wells were carefully bored into each agar plate after standing for about 5 minutes with heat sterilized 6 mm diameter cork borer and labeled. The first four wells were filled with 0.1 ml volume each of the 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml concentrations of the extracts prepared with 10% DMSO. The other two wells were filled with 0.1 ml of 4 mg/L ciprofloxacin as positive control and 10% DMSO as negative control. Care was taken not to allow spillage of the solutions onto the surface of the agar. The plates were allowed to stand for about 30 minutes for proper diffusion of the solutions before being incubated at 37°C for 24 hours. This experiment was conducted in duplicates. After 24 hours, antibacterial activity was evaluated by measuring the diameter of the zones of inhibition produced by the extracts against the test organisms in milliliters.

Determination of Minimum Inhibitory Concentration (MIC) of Plant Extract and Antibiotics [80-82]

The macrobroth dilution method according to CLSI was used to determine the MIC of the methanol extract of *A. sativum* and antibiotics against MDR isolates of *S. aureus*. Different concentration ranging from 1.563-200 mg/ml of the plant extract and 0.0156 -128 µg/ml of each antibiotic (ceftazidime, cefuroxime, erythromycin, cloxacillin and ciprofloxacin) were prepared by two fold serial dilution in nutrient broth.

Combination of different concentrations ranging from 0.25 – 2 x MIC of the extract and antibiotics were used to determine the MIC of their combination effect. The range of extract and antibiotic concentration used in this study was chosen so that the MIC of the extract and antibiotic will fall within the dilution range. Each tube was inoculated with 0.1 ml each of the standardized bacterial isolates. An un-inoculated nutrient broth tube was used as control. The tubes were incubated at 37°C for 24 hours. The MIC was taken as the lowest concentration of the methanol extract, antibiotic or their combination that showed no visible growth after 24 hours incubation at 37°C. The combination analysis was carried out in duplicate.

Combination Studies (The Checkerboard Method) [83]

The antibacterial activity of the combination of methanol extract of A. sativum and antibiotic (Ceftazidime (Caz), Cefuroxime (Cxm), Erythromycin (Ery), Ciprofloxacin (Cip) and Cloxacillin (Clx) against MDR isolates of S. aureus were investigated using the checkerboard method previously described by Jayaraman *et al.* [73].

The Fractional Inhibitory Concentrations (FICs) were derived from the MIC of extract alone, MIC of antibiotic alone, and MIC of extract plus antibiotic combination permitting no visible growth of the test organism after incubation at 37°C for 24 hours.

The FIC values for the combinations were calculated using the following formula:

FIC (antibiotic) = (MIC of antibiotic in combination with extract / MIC of antibiotic alone).
FIC (extract) = (MIC of extract in combination with antibiotic / MIC of extract alone).

The interactions between the antibiotics plus extracts were evaluated using the FIC indices which were calculated using the formula;
FIC Index = ΣFIC = FIC (antibiotic) + FIC (plant extract).
Combinations were classified as synergistic, if the FIC indices were ≤ 0.5, additive/indifferent if the FIC indices were > 0.5 – ≤ 4.0 and antagonistic if the FIC indices were > 4.0 .

Time-Kill Assay [83-85]
Time-kill assay was performed using the identified synergistic combinations by the checkerboard method. The assay was carried out on methanol extract of garlic (MEG) plus ceftazidime and MEG plus erythromycin against a single *S. aureus*. The macrobroth dilution technique described by Olajuyigbe and Afolayan, [84] with little modification was used in this analysis.
The extracts and antibiotics were incorporated into 10 ml nutrient broth in a screw-capped test tube at a concentration of ½ x MIC. The tubes containing the individual antibiotic, extract and their combinations were inoculated with 1.0 ml of the bacterial cultures diluted to x108 cfu/ml. Growth control tubes consists of extract/antibiotic free nutrient broth inoculated with test organisms. Immediately after inoculation, aliquots (0.1 ml) of the control tube was taken, serially diluted in sterile normal saline and plated on nutrient agar in order to determine the zero hour counts. The inoculated tubes were then incubated in a shaking water bath at 37°C for 24 hours. Aliquots of 0.1 ml of the culture were withdrawn at 0, 4, 8, 12 and 24 hours of incubation and serial 10-fold dilutions were prepared in sterile normal saline and the dilutions plated on nutrient agar in duplicates. After incubating at 37°C for 24 hours, emergent bacterial colonies were counted and compared with the culture control without extract or antibiotic. The results were expressed in log10 cfu/ml. Synergy was defined as decrease of ≥ 2 log10 cfu/ml in colony counts [83] and bactericidal activity defined as a ≥ 3 log10 cfu/ml (99.9%) decrease in the bacterial counts for each of the indicated times [85].

CONCLUSION:
One of the most potent and active component in garlic is the Sulphur compound called allicin which is a chemical compound produced when garlic is chopped, chewed or bruised. It is a powerful antibiotic and an agent that helps the body to inhibit the growth and development of pathogenic microbes. Ginger has direct antimicrobial activity and thus can be used in treatment of bacterial infections. Ginger belongs to *Zingiberaceae* family. The *Zingiberaceous* plants have strong aromatic and medicinal properties and are characterized by their tuberous or non tuberous rhizomes. Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. In many countries including Bangledesh, ginger is used in boiled food preparation. Staphylococcus aureus is the most medically important member in terms of pathogenicity of the group. Two other less important members are Staphylococcus epidermis and Staphylococcus saprophyticus.

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