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Comparative Analysis of Antibacterial Effect of Ethanol Extracted *Allium sativum* (Garlic) Oil and Ampicillin on *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*

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Abstract: Antimicrobial effect of the oil extracted from the bulbs of Allium sativum (garlic) purchased from the "Oje" market of Edeland in Osun State, Nigeria was assessed. The oil was extracted by Soxhlet extraction method using ethanol as extraction solvent. The extract was screened against five clinical microorganisms; Escherichia coli, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus in-vitro, using agar-well-diffusion method and Ampicillin as control. The extracted oil is yellowis h in colour with a pungent smell. Using different dilution concentrations of the extract, the highest zone of inhibition was recorded against B. subtilis 27±1.2 while P. aeruginosa showed least sensitivity at 8.0±00 at 100%. At 75% dilution, the highest zone of inhibition was 20±1.2 against E. coli while the least was against P. aeruginosa 13±1.4. At 25%, highest zone of inhibition was 18±00 against B subtilis and least 11±1.2.The effect of ampicillin ranges between 24±1.4 and 16±1.4 against the tested microorganisms. The ethanol extract of allium sativum showed more sensitivity to B, subtilis, at 100%, and E.coli, at 100%, 75%, and 25%, compared to Ampicillin.

Keywords: Antibacterial, Ampicillin, Extract, Garlic.

INTRODUCTION

The use of medicinal plants and its products to treat infections is an age-long practice, in Africa. Medicinal plants are important part of our natural wealth where they serve as important therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and conventional medicines (krishnaiah *et al.*, 2009). Many indigenous plants in Nigeria are used in inhibiting and curing various life threatening infections or diseases caused by pathogenic microorganisms. Medicinal plants are invariably used to source for new drugs, many drugs which are in use today were either obtained from plants or developed using plants chemical structure as templates (Ajaiyeoba *et al*., 2006). The importance of locally available plants as medicine for treatment of infectious diseases cannot be underestimated (Oladeji, 2016). It is therefore significantly important to examine the phyto-chemical properties and antimicrobial effects of some medicinal plants commonly used in Nigeria.

Interest in plants with antimicrobial properties is being reviewed because of the emergence of resistance strains of disease causing microorganisms against some conventional antimicrobials drugs, (Hawassa & Sekota, 2016). The World Health Organization (WHO, 2001) estimated that the world population that uses medicinal plants to treat different forms of ailments and diseases is about 80% and this value is even much higher in African. Some plant produce essential oils, valuable chemical compound which are considered the chemical weapons. These compounds may dissuade insects or protect the plant against bacterial and fungal attacks while they as well act as instrument to attract and seduce their pollinators (Hamid *et al*., 2011). He further stated that these volatile liquid's aromatic compounds may be found in different parts hitherto in the leaves (oregano), seed (almond), flower (jasmine), peel (bergamot), berries (juniper), rhizome (galangal ginger), root (angelica archangelica), bark (sassafras), wood (agar wood), resin (frankincense), petals (rose), and bulb (garlic).

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 African countries where it is used in traditional medicine for

infectious disease and some other cases (Daka, 2011). It is an annual plant with superficial adventitious roots, bulbs composed of a disk like stem (Sofoworo, A., 1982). Garlic played important dietary and medicinal role for centuries where it is commonly used in food as flavoring agent and when consumed in food it does not produce any obvious side effects (Padiya and Banergee, 2013). It was reported that *Allium sativum* as a medicinal plant portray some anti bacterial effects against some organisms but it is under-utilized due to its odour and taste, although, garlic is still being employed in folk medicine all over the world for the treatment of a variety of diseases (Ali, Thompson and Afzal, 2000). The prophylactic and therapeutic effects of garlic are ascribed to specific oil and water soluble organo-sulfur compounds, which are responsible for its typical odor and flavor (Mims *et al.*, 2021). Venegas *et al.* (2016) also reported the garlic bacterial inhibitory effect on species of *Aerobacter, Aeromonas, Bacillus, Citrobacter,Clostridium, Enterobacter, Escherichia, Klebsiella, Lactobacillus,Leuconostoc, Micrococcus, Mycobacterium, Proteus, Pseudomonas, Salmonella, Shegilla, Staphylococus, Streptococcus and Vibrio.*

Garlic act as an anti-bacterial agent that inhibits the growth of infectious agents while at the same time protect the body from pathogens, an example is the deadly *Bacillus anthracis* which causes the disease anthrax (Wolde *et al*, 2018). Dini, Fabbri and Geraci (2011) *Mycobacterium tuberculosis*, a pathogen that causes tuberculosis showed successfully sensitivity to several sulphur components found in Garlic. More so, Garlic consumption was reported to have significant effects on lowering blood pressure, prevention of atherosclerosis, reduction of serum cholesterol and triglyceride, inhibition of platelet aggregation, and increasing fibrinolytic activity (Kumar *et al*., 2013). Furthermore, Leyla, Peir and Ali, (2014) stated that in *in-vivo* animal experiments, intravenous administration of garlic extracts produced slight reduction in both systolic and diastolic pressures and oral ingestion of garlic extract in hypertensive animals brought the blood pressure back to the normal level (Marmitt, Goettert and Rempel, 2021).). In addition, the preventive effect of garlic on atherosclerosis has been attributed to its capacity to reduce lipid content in arterial membrane (Yu-Yah and Liu, 2001). It was also reported that garlic decreases the risk of peripheral arterial occlusive diseases, plasma viscosity, and unstable angina and increases elastic property of blood vessels and capillary perfusion (Allison *et al*., 2012). More recently, garlic has been proven to be effective against a plethora of gram-positive, gram-negative, and acid-fast bacteria. These include *Salmonella and Escherichia coli* (Adler and Beuchat, 2002). O'Gara *et al*. (2000) documented that garlic shows differential inhibition in beneficial intestinal micro-flora and potentially harmful entero-bacteria which includes *Pseudomonas, Proteus, Staphylococcus aureus, Escherichia coli, Salmonella, Klebsiella spp., Micrococcus spp., Bacillus subtulis, Clostridium spp., Mycobacterium spp,.* and *Helicobacter spp.*

The exploitation and general acceptance of plants of medicinal importance, like A*llium sativa*, is poorly inclined among the elite and the continuous use of chemical based orthodox medicine to fight against pathogenic bacteria has proven to have short or long term effects with the emergence of antibiotics resistant strains which is evident globally. These necessitated the need for alternative antibiotics that is new, natural, plant based, non-toxic, cost effective with minimal or no side effects to eliminate the menace of orthodox medicine. This research focused on the extraction of oil from the bulb of natural garlic plant using ethanol solvents and accessing the antimicrobial effects of the extract on selected pathogenic microorganisms namely *Bacillus subtilis, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

The aim of this study is to affirm the antimicrobial sensitivity of garlic oil extract on some clinical microorganisms: *Escherichia. coli, Bacillus cereus, Bacillus Subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* while the specific objectives assessed physico-chemical component of *Allium sativa* (garlic) oil; assessed the antibacterial effect of garlic oil extract on the named microorganisms; and, compare the sensitivity effect of garlic oil extract with a known antimicrobial agent (Ampicilin).

MATERIAL AND METHODS

Collection of plant material and oil Extraction

Fresh samples of garlic bulbs (*Allium sativum*) were purchased from 'oje' market in Ede, Osun state. Aseptically, the cloves on the bulbs were peeled to obtain the edible portion which was diced into tiny bits, dried, ground to powder and stored for use appropriately. In extraction, 15g each of dried garlic powder was weighed and wrapped in a whatman filter paper. Two of the wrapped samples were placed in the thimble. 200ml of ethanol was transferred into the round bottom flask. The soxhlet apparatus was placed on the heating mantle and the ethanol was allowed to boil. The extraction process continued into condensation and the condensing unit was removed from the extraction unit. The oil was obtained after the solvent was evaporated by placing over a

water bath for about 2-3 hours under reduced temperature (50ºC) and refluxing at 70ºC to remove excess solvent.

Physico-chemical analysis of garlic oil extract

Saponification value of the garlic oil was estimated by the ASTM D464 titration method as described by Abdulhamid *et al.,* (2014). The refractive index of the crude oils samples were measured using an Abbe Refracto-meter at 30 ± 0.1 after calibration. The iodine value of the oils were determined using a mathematical relationship between refractive index and estimated iodine as being described by Perkins (1995) and reported by Amos *et al.,* (2013). And, the peroxide values of the extracted oils were determined using the standard method described by Akpan *et al.,* (2006).

Preparation of oil concentrates

The extracted oil (100% concentrate) was diluted into different concentration of 75%, 50% and 25%. The dilution was done with methanol. The 100% oil constituted the undiluted form of the oil. The 75% concentration of the oil extract was constituted using 0.75ml of the extracted oil and 0.25ml of methanol in a sterile test tube. The 50% concentration of the oil was constituted using 0.5ml of extracted oil and 0.5ml of methanol in a sterile test tube while the 25% oil concentrate was constituted using 0.25ml of the extracted oil and 0.75ml of methanol in a sterile test tube. The extracted oil at different concentration was stored in a refrigerator at 40ºC for further use.

Test organisms

Test microorganisms used were sub-cultured from clinically important microorganisms collected from the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos Nigeria. The organisms used were *Bacillus cereus* ATCC 10872, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, and *Escherichia coli* ATCC 25922.

Preparation of media

Mueller Hilton agar (MHA) was used for the antimicrobial assay using agar well diffusion method while Nutrient broth was used for sub-culturing test organisms. The media were prepared according to manufacturer's specification. The glass wares used for media preparation were properly washed and rinsed with distilled water and dried-sterilized in the hot air oven at 160ºC. Media were sterilized by autoclaving at 121ºC for 15 minutes.

Antimicrobial susceptibility testing

The screening was done using agar well diffusion method as described by Harold (2002). Plate culture of 18-hour old broth culture of test organisms was made using pour plate method on a well labeled Mueller Hilton Agar plates using sterile swab and duplicated appropriately in an aseptic manner. Holes were made on the solidified nutrient plates containing the test organisms using a 6mm cork borer. The essential oil in different concentration/dilution was dispensed into the bored holes (100%, 75% and 50% concentrations) while negative control (methanol) and positive control (Ampicillin) dispensed into their appropriate holes. The inoculated plates were incubated at 37ºC for 24hours. The resulted clear zones inhibition in millimeters were measured, recorded and inhibitive effects of garlic oil extract estimated.

RESULTS AND DISCUSSION

The physico-chemical features of the extracted oil is presented in Table 1 while Table 2 shows the antimicrobial activities of garlic oil extract against different strains of clinical bacterial.

Table 1: Physico-chemical properties of ethanol extract of *Allium* sativa (garlic) oil.

Parameters	**Concentration unit**
Specific gravity	0.89 g/ cm^3
Moisture	8.95%
Saponification value	158.1 mg (KOH)/g
Iodine value	11.9 mg/100g
Free fatty acids	1.4±0.1%
Acid value	13.8 mg(KOH)/g

Peroxide value	215 mg/100g
Refractive index	0.97
Melting point	-12^{o}C
Colour	Light yellow
Odour	Pungent

Specific gravity of garlic oil was found to be 0.894g/ cm^3 which was lower than most edible oil such as sunflower oil, cotton seed oil and olive oil reported by Rahman and Lowe (2006) to be approximately 0.919g/ cm^3. The other features of the extracted oil was found to include:; 8.95% moisture, 0.97 refractive index, 11.9 iodine level, acid value (13.8), free fatty acids (1.4%), saponification (158.1) and peroxide values (215) respectively.

The oil from *Allium sativum* exhibited high activity against most of the organisms. The largest zone of inhibition was27 ± 1.21 recorded against *Bacillus subtilis* ATCC 6633 while the least zone of inhibition was 8 ± 0.00 recorded against *Pseudomonas aeruginosa* ATCC 9027 at 100% concentration. The activity of the oil at 75% concentration was significant against the bacteria species*; Escherichia coli* ATCC 25922 was highly inhibited at 20.± 1.2, followed by *Bacillus cereuss* ATCC 6633 at 18 + 1.06 while 15 ± 1.05, 13 ± 1.41 and 15 ± 4.24 was recorded against *Bacillus subtilis* ATCC 10872, *Pseudomonas aeruginosa* ATCC9027 and *Staphylococcus aureus* ATCC25922 respectively. At 25% concentration, zones of inhibition of 18 ± 0.00, 12 ± 1.41; 13 ± 0.00 11 ± 1.2 and 14 ± 0.00 was recorded against *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10872, *Escherichia coli* ATCC 25922, *Pseudomona aeruginosa ATCC 9027* and *Staphylococcus aureus* ATCC 25923 respectively.

Table 2: Antimicrobial effect of ethanol extract of *Allium* sativa (garlic) oil

Microorganism	**Zone of inhibition (mm)**			
	Essential oil 100%	**Essential oil 75%**	**Essential oil 25%**	**Ampicillin**
Bacillus subtilis ATCC 6633	27 ± 1.21	15 ± 1.05	18 ±0.00	20 ± 0.00
Bacillus cereus ATCC 10872	13 ± 1.00	18 ± 1.08	12 ±1.41	22 ± 4.24
Escherichia coli ATCC 25922	21 ± 1.50	20 ± 1.20	13 ± 0.00	16 ± 1.41
P. aeruginosa ATCC 9027	8 ± 0.00	13 ± 1.41	11± 1.20	16 ± 2.83
S. aureus ATCC 25922	22 ± 2.83	15 ± 4.24	14 ± 0.00	24 ± 1.41

The garlic oil at 100%, 75% and 25% showed good activity against bacteria species when compared with the standard antibiotic ampicillin.

The result of the antimicrobial effect of the ethanolic extract of *Allium sativum* revealed the largest zone of inhibition of 27 ± 1.21 against *Bacillus subtilis* ATCC 6633 at 100% concentration which was better than the effect of ampicillin against the microorganism. Also, the oil extract was more sensitive to *Escherichia coli* ATCC 25922 compared to Ampicillin. Daka (2011) reported that extract of garlic showed antibacterial activity against *Staphylococcus aureus in vitro.* In addition, Manu, Rachna and Nidhi (2019) study also suggested that garlic have anti -bacterial activity against *Staphylococcus aureus* and *Escherichia coli* and can be used against pathogenic microorganisms.

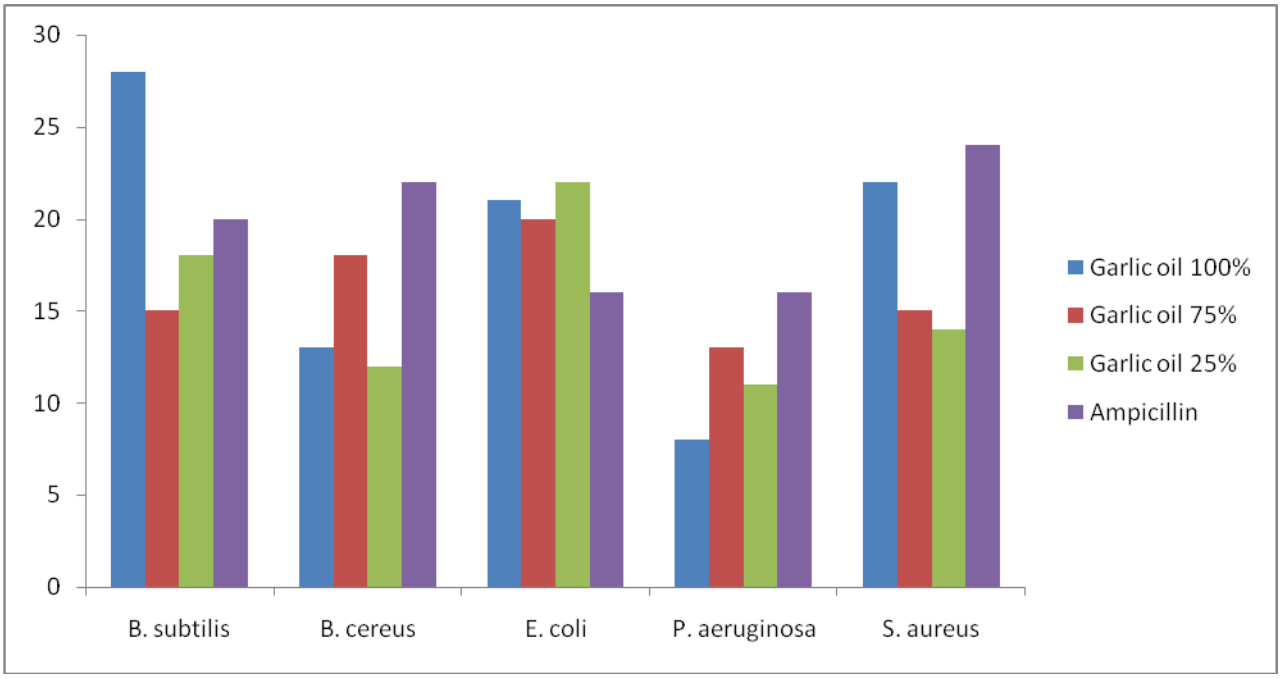

Figure 1: Comparative chart showing Antimicrobial effects of ethanol extract *Allium sativa* (garlic) oil and Ampicillin

The oil extract was more sensitive against *E.coli* at all concentration compared to Ampicillin while *B. subtilis* showed better susceptibility at 100% concentration compared to Ampicillin. Whereas, other organisms *B. cereus, P. aeruginosa* and *S. aureus* were less susceptible to the oil extract at all concentration compared to Ampicillin. Onyeagba reported a contrary result that crude extracts of garlic applied singly did not exhibit any *in vitro* inhibition on the growth of test organisms including *Staphylococcus* spp. (Onyeagba, 2004). But, Hamdy, Soad and Doaa (2016), reported that garlic essential oil extracts exhibited different inhibition levels against different tested bacteria. Also, Daka (2011) reported that oil extract of garlic have concentration dependent antibacterial effect against *Staphylococcus aureus.*

The result showed that extracted oil from the bulbs of *Allium sativum* (garlic) exhibit significant activity against tested microorganisms *Bacillus subtilis, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*, although, with different level of severity. The inhibitory effect of the essential oil at different percentages (100%, 75%, and 25%) showed relatively high zones against *B. subtilis, B. cereus, E.coli* and *S. aureus* except for *Pseudomonas aeruginosa* whose zone of inhibition was comparably very weak compared to the standard Ampicillin. In the report by Hamdy, Soad and Doaa (2016) it was also affirmed that garlic oil extract exhibit variant level of antimicrobial activity against different species of microorganism and it further declared that garlic oil extracts exhibited marked inhibition activity against bacteria at high concentrations. The findings suggested that the garlic oil extracted with ethanol solvents showed appreciable antimicrobial activities against *Bacillus cereus, Bacillus subtilis, Escherichia coli, Psuedomonas aeruginosa* and *Staphylococcus aureus.* The ethanolic oil extracts had more inhibitory effect on *E.coli* at 100%, 75% and 25% dilution while *B. subtilis* was more susceptible to the *allium sativum* oil at 100% dilution compared to Ampicillin. Study by Elias *et.al.* (2022) showed that significance differences were recorded in the inhibitory effect of garlic oil extracts at different concentration levels against different bacteria.

CONCLUSION AND RECOMMENDATION

In conclusion, ethanolic extract garlic oil have antibacterial potential and could be used as an alternative to common antibacterial drugs in the treatment of some common bacterial infections. Albeit, expanded research into this subject could lead to more discovery. It is recommended to carry out further trials to establish the clinical efficacy, safety, antioxidant effect and toxicity of the extracted garlic oil.

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