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Antimicrobial Activity of Garlic and Honey on *Staphylococcus Aureus* and *Escherichia Coli*

David Ejovwo Avwunugbe[1], Olivia Sochi Egbule[2*], Omenogor Patricia[3], Konye, Olubunmi Olufunmi[4]

[1,2,4]Department of Microbiology, Delta State University, Abraka

[3]Department of nursing science, Delta State University, Abraka

ABSTRACT

The numerous side effects of conventional antibiotics and increasing antibiotics resistance of some strains of bacteria such as *Staphylococcus aureus* and *Escherichia coli* which are commonly implicated in skin infection, surgical site infections, urinary tract infections (UTIs), neonatal meningitis, systemic infections and enteric infections in humans makes it is pertinent to source for alternative remedies.

Aim: To assess the antimicrobial activity of honey and garlic on *Staphylococcus aureus* and *Escherichia coli*

Methodology: Standard microbiological procedures were employed to isolate and identify the bacteria species. Antimicrobial activity was determined using the agar well diffusion method.

Result: The *S. aureus* and *E. coli* were susceptible to the honey and garlic extracts. *S. aureus* was susceptible to garlic, honey and the mixture of both with zones of inhibition ranging from 6.9-17.2mm, 4.8- 12.9mm and 6.0-17.0mm respectively while those of *E. coli* ranged from 5.8-16.4mm, 5.0-11.8mm and 5.6-16.0 respectively. There was significant difference ($p \leq 0.05$) in the antimicrobial activity of the garlic, honey and the mixture.

Conclusion: This study therefore reveals that honey and garlic possess significant antimicrobial activity against the tested organisms with high antibacterial activity occurring at high concentrations. Honey and garlic can therefore, serve as substitute to antibiotics.

KEYWORDS: honey, garlic *Staphylococcus aureus. Escherichia coli*, urinary tract infections

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INTRODUCTION

Infectious diseases are of global concern because they are the leading cause of global morbidity and mortality. Deaths caused by bacterial infections accounted for more than 1 in 8 global deaths in 2019, with five pathogens accounting for more than half of those deaths. Bacterial infections were the second leading cause of death in 2019 behind ischemic heart disease and affected all age-groups. The estimated 7.7 million deaths associated with 33 bacterial pathogens constituted 13.6% of all global deaths in 2019 (Dall, 2022). The spread of infectious diseases results mainly from changes in human behavior including lifestyles and land use patterns, increased trade and travel, and inappropriate use of antibiotic drugs which has further caused mutations in pathogens (Egbule, 2016). *Staphylococcus aureus* and *Escherichia coli* which are part of the normal flora of the human body are also a major cause of several humans and animal infections (Egbule *et al*., 2016). *S. aureus* have been implicated in skin and soft tissues infections, surgical site infections. *Staphylococcus aureus* is also a common cause of nosocomial infection which include hospital-acquired bacteremia and hospital-acquired respiratory tract infections (Abulreesh and Organji, 2011). On the other hand, *E. coli* is common cause of urinary tract infections (UTIs), neonatal meningitis, systemic infections and enteric infections in humans (Kim, 2012; Foxman, 2010). However, both species are among the most frequent causes of bacteremia and sepsis in Western industrialized countries, with observed proportions of 16.3% to 21.6% for *S. aureus* and 5.6% to 24.2% for *E. coli* among all causes of sepsis, associated with considerable morbidity and mortality (Bhattacharya, 2018).

Infections caused by *Staphylococcus aureus* and *Escherichia coli* have being successfully treated with a wide range of antimicrobial agents. Major challenges encountered with antibiotics in clinical use are resistance to antibiotics which leads eventually to failure of the treatment (Blair, 2004). It is

also a well-known fact that the use of conventional antibiotics have being associated with adverse side effects ranging from mild rashes, nausea, dizziness to hypersensitivity as seen in penicillin, neutropenia, gastrointestinal disorder to severe effects such as auditory damage, kidney damage and problems of the central nervous system (Saimir and Llma, 2018).

However, in developing countries such as Nigeria, there is poor access to health facilities and lack of funds to purchase first-line antibiotics (Egbule and Yusuf, 2019). Herbal remedies and natural substances are being used as alternative medicine, as they are readily available and continue to play a major role in traditional setting as therapeutic remedies in many developing countries (Jonathan *et al.*, 2007). Problems of various antibiotics as stated above have promoted investigations into natural and potent antimicrobial substances. The therapies have drawn the interest of both public and medicinal communities. Current research has been focused on herbal and aromatherapy product. However, a number of such products include honey and garlic which has shown therapeutic promise (Jonathan *et al.*, 2007).

Honey is antibacterial and can prevent growth of *Acinetobacter baumannii, Streptococus species, Enterococcus cloacae* among many others. Honey has been used as a medicine since ancient times (Maeda *et al*., 2008). More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including gram-positives, gram-negatives, aerobes and anaerobes (Halawani and Shohayeb, 2011).

More so, the use of higher plants such as garlic and preparation from them to treat infections is an aged-old practice. Interests in plants with antimicrobial properties had come alive again because of emergence of resistance strains against antimicrobials such as penicillin. There is appreciable epidemiologic evidence that demonstrates therapeutic and preventive roles for garlic. Several experimental and clinical investigations suggest many favorable effects of garlic and its preparations (Colín-González *et al*., 2012; Aviello *et al*., 2009). Among the organisms which garlic has been reported to inhibit include *Bacillus anthracis,* oral *Streptococci* strain and *Staphylococcus sp.* Thus, this study was conducted to evaluate the antibacterial effect of garlic and honey on clinical isolates of *Staphylococcus aureus* and *Escherichia coli*

MATERIALS AND METHOD

Isolation of bacteria

This study was limited to the determination of antimicrobial assay on bacterial isolates from fifteen (15) skin swab of students randomly sampled for isolation of *Staphylococcus aureus* while stock culture of *Escherichia coli* obtained from diarrhea stool was collected from the Microbiology Laboratory, Delta State University, Abraka. The samples were inoculated under aseptic conditions on nutrient agar, blood agar (5% sheep blood), and MacConkey agar plates, incubated aerobically at 35°C–37°C for 24–48 h. Primary identification and characterization of isolates were performed on the basis of Gram staining, microscopic characteristics, colony characteristic, and secondary identification were done using biochemical tests such as triple sugar iron agar, hydrogen sulfide test, carbohydrate fermentation test, phenylalanine deaminase test, methyl red test, nitrate reduction test, urease test, Vogesproskauer, citrate utilization test, and indole test using standard microbiological methods.

Preparation of garlic extract

The garlic was cleaned, peeled and air dried for 14 days, thereafter it was blended using a sterile blender (Eurosonic - ES-1008). The powdered garlic was weighed to be 100g and poured into a conical flask containing 75cl ethanol, shake to homogenize and after 48hours the extract was obtained by filtering off the garlic sediment using filter paper. The filtrates were discarded appropriately.

Antibacterial sensitivity test

The antibacterial activity of honey that was collected from local honey vendors and the garlic extract was tested in-vitro using Agar well diffusion method (Kirby Bauer's method). The test material was prepared by diluting each honey in sterilized distilled water at different dilution (concentration) 25%, 50% also net honey (100%). Garlic extract at different concentration of 25%, 50% and 100%. Muller-Hinton agar plates were prepared and each plate was properly inoculated with each test organism using streaking method with the help of a sterile swab stick. Wells were drilled using a sterile cork borer and each well was filled with different concentration of the honey and garlic. The plates were incubated at 37^{o} C for 24 hours and observed for zones of inhibition.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the mean values as a measure of test of significance. A $P < 0.05$ was considered statistically significant**.**

RESULTS

Antimicrobial activity of the honey on *Staphylococcus aureus* and *Escherichia coli* is presented in Table 1 with *S. aureus* showing higher susceptibility than *E. coli*. The antimicrobial activity of the garlic on *Staphylococcus aureus* and *Escherichia coli* was found to be higher than that of honey with *S. aureus* being more susceptible. The zones of inhibition of the mixture of both garlic and honey on *E.coli* and *S. aureus* did not show any significant increase rather they were similar to the result of that of garlic as shown in Table 1.

Table 1: Zones of inhibition of different concentrations of garlic, honey and mixture (honey + garlic) on *S. aureus* and *E.coli*

Concentration (%)	zones of inhibition (mm)					
	Garlic		Honey		Mixture	
	E.C	***S.A***	***E.C***	***S.A***	***E.C***	***S.A***
100	16.4	17.2	11.8	12.9	16.0	17.0
50	11.8	11.8	8.6	7.3	12.0	12.0
25	5.8	6.9	5.0	4.8	5.6	6.0

Values are expressed as Mean for n=2 determinations, P value ≤ 0.05

Key

E.C= *Escherichia coli*

S.A= *Staphycoccus aureus*

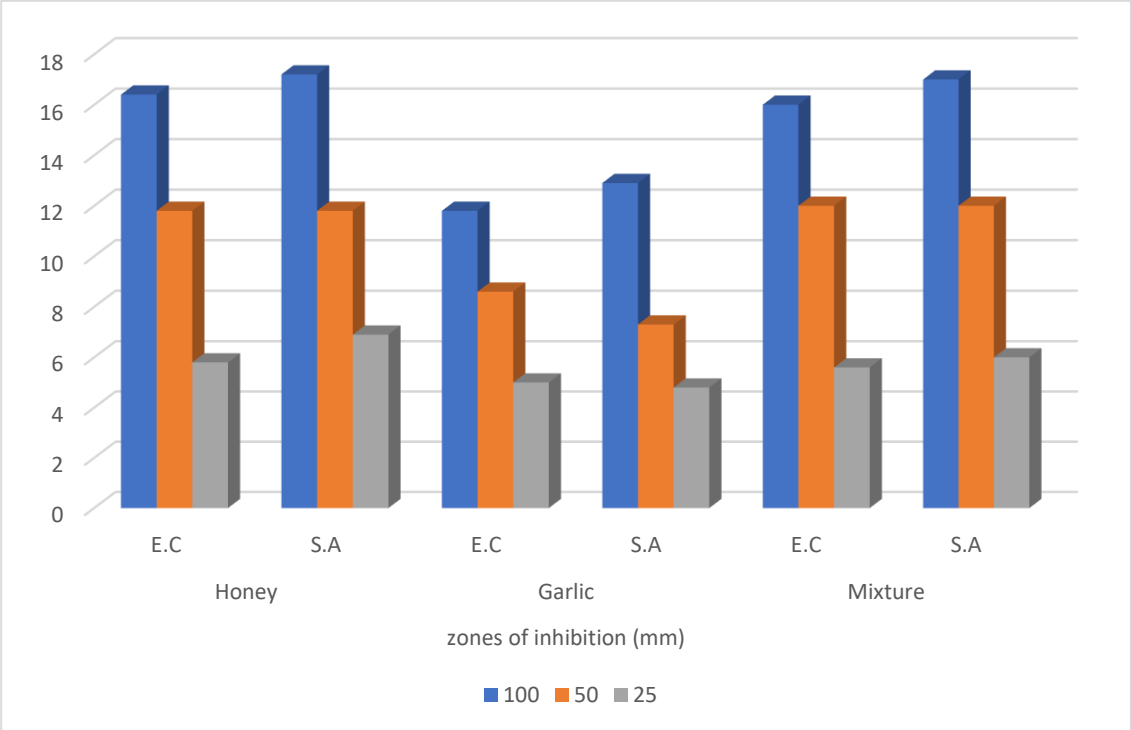

Figure 1: Antibacterial sensitivity test results

DISCUSSION

Different reports globally had demonstrated the antibacterial potential of honey and garlic extracts separately against different clinical isolates collected from wounds, UTIs, nosocomial infections and enteric pathogen (Abdullah *et al*., 2012; Mulu *et al*., 2004).

This research was carried out to investigate the possible antibacterial activity of honey and garlic on *Staphylococcus aureus* and *Escherichia coli* isolated from skin of Microbiology students. *Staphylococcus aureus* and *Escherichia coli* are normal flora of the body and have being implicated in infections ranging from sepsis, bacteremia, neonate meningitis, urinary tract infections, abscesses, peritonitis, diarrhea and opportunistic infections. Treatment with conventional antibiotics has numerous challenges such as development of resistance, adverse side effects, allergic reactions and high cost of the drugs. Hence, the evaluation of natural alternative to antibiotics is essential.

The antimicrobial activity of honey and garlic at varying concentrations of 100%, 50% and 25% exhibited significant zones of inhibitions on the *S. aureus* and *E. coli*. From the results, *S. aureus* was more susceptible than *E. coli* to both honey and garlic showing zone of inhibition of 12.9mm, 7.3mm and 4.8mm for a corresponding concentration of 100%, 50% and 25% for honey. There was greater susceptibility to garlic with zones of inhibition of 17.2mm,

11.8mm, and 6.9mm for a concentration of 100%, 50% and 25% respectively. Also, *E. coli* was susceptible to honey with zones of inhibition of 11.8mm, 8.6mm and 5.0mm at concentrations of 100%, 50% and 25% and a greater susceptibility to garlic with zone of inhibition of 16.4mm, 11.8mm, and 5.8mm at 100%, 50% and 25% concentrations respectively. This may be due to presence of phytochemical which have been found to be antimicrobial (Muley *et al*., 2018).

Comparing both results for honey and garlic, garlic showed a higher antimicrobial activity on both organisms. Different authors have reported that the antimicrobial activity of garlic is due to the allicin inherent in it which interferes the normal process of RNA production and lipid synthesis, which affects the synthesis of protein and cell wall of microorganisms. Futhermore, compounds present in garlic like adjoene, enzymes (peroxidase and miracynase), different amino acids such as cysteine, glutamine and methionine, vitamins B and C may also be responsible for its antimicrobial activity (Kumar *et al*., 2010). Honey's antimicrobial behavior is dependent on factors such as H_2O_2, pH, and low water activity, non-dissociated organic acid and Phenolic compounds (Harvesteen, 2013). Another report from Molan *et al*. (2018) also said that the floral source of which, the honey is made of contributes to the antimicrobial activity of honey. Lysozyme and another volatile compound present in honey may have roles in the inhibition of microorganisms (Bogdanov *el al*., 2008)

The zones of inhibition increased with the concentration of the honey and garlic. This result agrees with Wilix *et al.* (2009) who reported that honey and garlic possess significant antimicrobial activity against the tested organisms. The highest antibacterial activity was at their undiluted state and there was also variation in the potency as the concentrations of honey or garlic is reduced. The zones of inhibition for the mixture of both honey and garlic showed no significant difference from the result obtained from that of garlic. However, garlic showed higher antibacterial activity compared to honey with *S. aureus* being more susceptible than *E. coli*. Based on the findings of this research both honey and garlic showed antibacterial activity against *S. aureus* and *E.coli*. The result from this current research agrees with that of Muley *et al*., (2018) and that of other researchers. Mousa *et al*., (2012) carried out a research on different Algerian honey types, reported that honey inhibited the growth of *S. aureus* at 50% concentration. Abubakar (2009) also studied the antibacterial activities of different Saudi Arabian honey which came from different floral sources and compared their inhibition capacity against *S. aureus, K. pneumoniae* and *P. aeruginosa*. Their findings indicates that the different honey samples were effective against the tested pathogens at different concentrations (Omoya and Akhariyi, 2011). Similarly, in this study honey inhibited all tested pathogens at concentration of 25% v/v.

CONCLUSION

Honey, garlic and the combination of both successively inhibited the growth of *S. aureus* and *E. coli*. The combination of both honey and garlic showed no significant increase in the zone of inhibition however, the mixture of both was reflecting the result obtained from that of garlic. Based on the results of this study that shows honey and garlic successively inhibited *S. aureus* and *E. coli* they could be used as an alternative to conventional antibiotics.

RECOMMENDATION

It is recommended that further research should be done on both Honey and garlic to determine the concentration that would be sufficient to act in-vivo and use as a substitute. The addition of honey and garlic to diet is encouraged to reduce the occurrence of *S. aureus* and *E. coli* infections.

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Conflict of interest: The authors declare no conflict of interest.

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