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Anti-Bacterial Effect of Garlic (*Allium sativum*) against Clinical Isolates of *Staphylococcus aureus* and *Escherichia coli* from Patients Attending Hawassa Referral Hospital, Ethiopia

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Abstract

Introduction: 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.

Objective: This study 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.

Methodology: 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.

Results and conclusions: 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.

Keywords: *Staphylococcus aureus*; *Escherichia coli*; Garlic

Abbreviations: *E. coli*: *Escherichia coli*; EPHI: Ethiopian Public Health Institute; EHNRI: Ethiopian Health and Nutrition Research Institute; Lab: Laboratory; MRSA: Methicillin Resistant *Staphylococcus aureus*; MSA: Mannitol Salt Agar; Staph: *Staphylococcus*.

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Introduction

The use of higher plants and preparations from them to treat infections is an age-old practice. Interests in plants with antimicrobial properties has come to use again because of emergence of resistance strains against antimicrobials such as penicillin [1].

Garlic (Nech- shinkurt in Amharic/Local name/) (*Allium sativum* L.) is under family Liliacea. It is an erect annual herb with superficial adventitious roots, bulbs composed of a disk like stem [2]. It has long tradition as medicinal plant, started with a direction of preparing a medicinal remedy written in a cuneiform character in about 3000 BC. Scientific investigations of various garlic preparations began in 1939 [3].

There are a number of studies carried out to assess the value of herbal remedies including garlic preparations for treat of illness [1,2,4].

Recent studies in Ethiopia indicated that garlic has been commonly used in Ethiopian traditional medicine for infectious diseases like tuberculosis, sexually transmitted infections, wounds etc. Besides, its many other culinary applications [1,5].

The antibacterial effect of Garlic (*Allium sativum*) and other *Allium spps* has been attributed to *S. aureus* and *E. coli* [2].

This study confirmed that the aqueous extract of Garlic had antibacterial effect against clinical isolates of *S. aureus* and *E. coli.*

Literature Review

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 years 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 [6,7].

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 [7]. 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 hydyl (SH) groups, or thiols this finding has important implication because of sulfhydryl groups are also crucial component of some enzymes that participate in the synthesis of cholesterol "Garlic lowers the level of harmful cholesterol'' [7-14].

Review on test organism

Staphylococcus aureus is very important pathogen that causes a variety of diseases including skin infections. Gastro intestinal disease (*staph*) food poisoning, toxic shock syndrome and nosocomial infections acquired during hospitalization.

Staphylococcus aureus is isolated on Mannitol salt agar which is a medium that is high in salt (75% NaCl) and contains Mannitol as a source of carbon and energy. *S. aureus* ferments the mannitol and causes the medium turn yellow.

Escherichia coli is a bacteria that exists naturally as part of normal gut of healthy humans and mammals. It causes enteric diseases. However, there are relatively few strains of this strains pf this organism, which are pathogenic to humans and are associated with food related illness [10].

Emergence of Methicillin Resistant Strains of *S. aureus* (MRSA)

There is a growing medical problem due to increasing frequency of infection caused by penicillin resistant S*taphylococci*. B-lactamase producing strains of *S. aureus* that are resistant to penicillin first appeared in clinical specimens in early 1950s. Soon thereafter, multiple antibiotic resistance was detected in chemical isolates of *S. aureus*; these strains was resistant to macrolide antibiotic, amino glycoside, and tetracycline.

Plasmids and transposons are clearly important in conferring and transferring antibiotic resistance between bacteria.

Although plasmids and transposons are certainly involved, the actual evolutionary mechanisms underlying this phenomenon have yet to be explained. One this consequence is the emergence of epidemics hospitals strains of *S. aureus* that is resistant to virtually all useful antibiotics, including methicillin and vancomycin. These strains are currently a significance cause of nosocomial (hospital acquired) infections in parts of the world [11].

Ethical clearance

Ethical clearance was obtained from Ethical board of Debub University and support letter were delivered to Ethiopian Public Health Institute and Hawassa Referral Hospital.

Materials and Methods

Plant materials

Garlic solution was obtained not from the whole part of the plant rather on the bulbs. The bulb of Garlic were peeled, weighted and then ligated using pestle while adding small amount of H_2O. The extracts then allowed to freeze at -18°C (deep freezing) so as to concentrate the chemical (allicin). Then after freezing, the filtrate was put into lyophilizer till an amorphous powder weighted and then diluted with distilled water and used for the experiment [12].

Test organism

Two test organisms (*S. aureus* and *E. coli*) which were clinically isolated from patients were collected from Awasssa Referal Hospital.

Two standard pure cultures were collected from EHNRI (Ethiopian Health and Nutrition Research Institute). Both clinical and standard were sub cultured into subsequent Nutrient broth and definite Medias in which they favored i.e., Mannitol salt agar (MSA) for *S. aureus* and Mackonkey for *E. coli* were prepared on a slant and on petri dishes [10].

Inoculum

Cultured of the test organisms were maintained on nutrient both. Briefly, four to six colonies were picked with an inoculating loop and suspended in 5 ml of broth and incubated at 37°C for 24 hours. The turbidity of the broth culture was then equilibrated to match that of 0.5 Macfarlands standards. This provides organisms in the range of 1×10^6 to 5×10^8 cfu/mol which is pathogenic that used for the test [12].

Antibacterial activity test

The antibacterial activity test of the crude extract of Garlic against both standard and clinical isolates were carried out by the Agar-diffusion method [13].

Agar diffusion method

The molten agar will mixed with a different concentrations of the test samples at molten state 45-50°C and mixed aseptically with different amounts of garlic extracts to a concentration of 0.25 ml, 0.5 ml, 0.75 ml and 1.5 ml which is equivalent to 5 mg/ml, 10 mg/ml, 15 mg/ml and 25 mg/ml of media. Then, the prepared media were let to solidify. A separate agar plate without sample or drugs was also prepared in order to provide an appropriate growth of organisms. (As the same time as control) Two standard drugs as a positive control were also tested against these micro-organisms. These were chloramphenicol 0.30 mg and penicillin 0.30 mg. The negative control used in the cork borer as well as the solvent i.e., distilled water.

Antibacterial effect was determined by direct visual comparison of the growth of the test cultures. All the tests were carried out in triplicate and the results were reported as the averages of these replications.

Cork borker method

In this methods 0.2 ml of garlic extract was mixed with 20 ml of sterile nutrient agar using a mixer (vortex), and then poured into sterile Petri dishes. After congealing, the seeded agar was punched out with a sterile bore (back hole of 10 ml pipette diameter=9 mm) at equally spaced out positions to make four holes. Four of the holes were filled with 0.1 ml of the test sample solution while the fifth with standard antibiotics (chloramphenicol+distilled water) per hole. The plates were then left at room temperature for 2 hours (to favor diffusion over microbial growth) and incubated in an incubator at 37°C for 24 hours. As mentioned before each sample done in triplicate.

The antibacterial activity was evaluated by measuring the diameter of the zone of inhibition using ruler (the media in both methods was prepared according to the instructions that the manufacturer orders (written in the flask that for how many gm of powder how money ml of distilled water is enough).

Results

The results of the susceptibility of the test organisms against the garlic extracts which are stated below in **Tables 1a and 1b. Table 2** showed that both clinical isolates of *S. aureus* and *E. coli* were sensitive to the concentration of 15 mg/ml (0.75 ml/20 ml of agar media) which is about 80% but about 10% of the organisms were not sensitive for lower concentrations i.e., for 0.25 ml in media.

Table 1a Result of Antibacterial activity of Garlic aqueous extract by agar diffusion method.

Isolates	Micro organisms	Concentration of Garlic/20 ml			
		0.25 ml	0.5 ml	0.75 ml	1.5 ml
Clinical	*S. aureus*	-	+	+	+
	E. coli	-	-	+	+
Standard	*S. aureus*	+	+	+	+
	E. coli	+	+	+	+

Table 1b Result of antibacterial activity of Garlic extract compared with standard drugs against test organisms by Agar diffusion+indicates Inhibition-indicates growth.

Antibiotic type	Concentration	Size of Clear zone	Organisms presence or absence	
			S. aureus	*E.coli*
Garlic	5 mg/ml	Small	-	-
	10 mg/ml	Small	+	-
	15 mg/ml	Medium	+	+
	25 mg/ml	Large	+	+
Tetracycline	30 mg/ml	Very small	-	+
Chloramphenicol	10 mg/ml	Large	+	+
Penicillin	30 mg/ml	Medium	-	+

Table 2 Result of antibacterial effect of Garlic extracts compared with standard drugs against test organisms.

Antibiotics	Concentration (per 20 ml of media)	Diameter of Clear zone (in millimeters)			
		Clinical Isolates		Standard Isolates	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
Garlic extracts	5 mg/ml	13	11	13	10
	10 mg/ml	15	11	16	13
	15 mg/ml	20	16	24	17
	25 mg/ml	24	19	28	26
Tetracycline	30 mg/ml	10	10	22	16
Chloramphenicol	10 mg/ml	24	17	32	25
Penicillin	30 mg/ml	11	13	20	14

In addition, larger clear zones were observed at higher concentrations against both microorganisms. Comparatively, Garlic extracts inhibited bacterial growths than Tetracycline and Penicillin.

Discussion

The result showed the both clinical and standard isolates of *S. aureus* and *E. coli* were highly sensitive to concentrations of 0.75 ml/20 ml of agar media in using diffusion method and Cork borers. Moreover, unlike clinical isolates of *S. aureus,* clinical isolate of *E. coli* was a bit resistant/not sensitive/ at concentration of 0.5 ml/28 ml of media. This could be in regard with the nature permeability of *E. coli*, which means 20% of membrane of *E. coli* is made of lipid while that of *S. aureus* is only made of 2% lipid [2]. Therefore, the garlic extract was more important for the prevention of resistant *S. aureus* which is currently becoming a challenge developing resistance to many commercially available drugs like penicillin.

In this study we have observed that, as the concentration of the garlic extract increases we have seen efficiency increased and hence inhibition and growth of test bacteria has been diminished. As observed from the above tables, Larger clear zones at higher concentrations and lower clear zones at lower concentrations. This implies that, Garlic has both bacteriostatic and bactericidal effect.

Conclusion

Based on the results of this study which showed garlic to make large clear zones than currently available antibiotics used in the study Garlic could be used as an effective antibacterial agent in Ethiopia where *S. aureus* is known to be resistant.

It could be made as a tablet in the best concentrations and affordable dosages so that it can be used as medicine to these two pathogenic gastro intestinal enteric. In the era of those drug resistant bacteria, we need to focus on alternative drugs that have long history to avoid such emerging diseases and that could be easily available and affordable.

Recommendations

One can use garlic as a member of the daily diet for better health especially the fresh garlic. This research can be used as a base to well identify the actual allicin minimum inhibition concentration.

Consent to Publish

Not applicable.

Competing Interests

The authors declare that they have no any competing interests.

Author's Contribution

EA and AB designed the study methodologies and EA carried out the investigation and AB edited the manuscript. All authors approved the manuscript.

Availability of Data and Materials

Materials used for the study were stored in Hawassa University, Department of Biology Laboratory. But the files are fully presented in this manuscript.

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