Evaluation of the Antibacterial Activity of Iraqi Garlic Derivatives to Escherichia Coli

Minen Al-Kafajy, PhD

Lecturer, Microbiology department , college of Medicine, Thi-Qar University, Iraq

ABSTRACT

Objectives: As a foodborne pathogen, Escherichia coli (E. coli) encounters many barriers to invade and disseminate in the human body, but it might cause disease. Although Fresh garlic juice (GJ) and Garlic oil (GO) were reported to possess antibacterial activity, the mechanism that underlay the garlic antimicrobial activity still obscure.

Method: The hypothesis of my study is to examine the antimicrobial activities of GJ and GO and the combination of either GJ or GO with Amp and CF, respectively.

Results: In my current project, the antibacterial activity of GJ was found to be significant in reducing the bacterial protein synthesis against E.coli, while GO negatively correlated; but, unfortunately, did not reach the significant level. Moreover, GJ and GO were correlated with improvement of the sensitivity of bacteria to antibiotics.

Conclusion: further investigation of the potency of GJ and GO in treating the infections is highly needed.

Keywords: *E. coli, antibacterial activity, garlic juice, garlic oil*

Introduction

Historically, Garlic (Allium sativum) has considered as customary dietary and medicinal supplements as an anti-infective agent [1]. In vitro, it has been proofed the antimicrobial action of fresh and freeze-dried garlic extract against numerous microorganisms such as viruses [2] Bacteria [1] and fungi [3]. It has been reported in several papers that allicin (diallyl thiosulfinate) is the primary inhibitory component of garlic that possess anti-microbial activity [4].

Several papers have reported that the main inhibitory component of garlic is the allicin (diallyl thiosulfinate) that produced when garlic cloves are compressed. The concerning issue in the allicin is unstable, and this could be the reason in the differences in reporting garlic bioactivity in multiple publications [5]. Interestingly, Cavallito and his workers were discovered that removing allicin from the garlic juice will demolish its biological activity [6].

On the other hand, E.coli is one of the few microorganisms that considered versatile discovered for the first time by Theodor Escherich in 1885. It is one of the harmless bacteria residing in the intestinal area of the human and mammals. As a healthy flora, it inhabits in a mutually beneficial relationship with the host and seldom causes illness. In the industry, E. coli has been widely used in recombinant DNA technology to clone various types of plasmid, produce proteins, nucleic acids, etc [7].

Despite the harmlessness of E.coli, it could cause a severe infection that may affect humans and feeding mammals. E.coli rod-shaped belongs to a gram-negative facultative aerobic bacterium Enterobacteriaceae family historically discovered in the soil and rotting vegetation. Some of the E.coli strains obtaining virulence factors throughout plasmids, transposons, bacteriophages, and pathogenicity islands. Although only seven serotypes of E.coli are recognized as pathogenic, thirteen serotypes of E.coli have been discovered, but only seven of them causing most dangerous cases of human disease such as

Corresponding Author:
Minen Al-Kafajy
Department of Microbiology, Thi-Qar University, Thi-qar, Iraq
Email: minen2006@gmail.com

dysentery, diarrhea, and infections in the area other than the intestine such as the urinary system and meninges [7].

Studies that focused on using a fecal carriage estimated that most people will consume E. coli contaminated foods between 10 and 15 times a year in middle east countries. E. coli is a common pollutant of a raw diet, nuts, and dairy, and is yearly blamable for many universal recalls of both human and animal food. The USA Centers for Disease Control and Prevention (CDC) has estimated that the epidemic cases were associated with consumption of food products, 73,000 illnesses, 2,200 hospitalizations, and 60 deaths annually in the United States. Complete genome sequencing of bacteria isolated from the product confirmed the genetic presence of the E. coli in the isolated bacteria. In recent years, E. coli accounted for a stunning 50% of the total amount of food recalled by the USDA, the liability in large part to contamination of frozen vegetable products manufactured by food companies. [8]

For the majority of humans who ingest E. coli, the only symptoms that happen will be self-limiting gastroenteritis or flu-like symptoms. In the senior people, however, or in patients with low immunity level such as HIV, cancer, diabetes mellitus, and those on immunosuppressive medicine, the disease may proceed to invasive enteric illness, described by septicemia, meningitis, and encephalitis, before analysis is made. The latest study ranked E. coli infection as the fifth most costly foodborne pathogen in the US because the invasive enterobacteria generally result in admission to intensive care [9]STEC were isolated from 30 (43%.

Fortunately, E. coli is susceptible to a wide range of antibiotics, including trimethoprim-sulphamethoxazole and β-lactams, and provided that proper treatment is initiated early; many who are diagnosed will recover. Once the bacteria have crossed the blood-brain barrier, however, management becomes more arduous, and consequences are generally less favorable. In humans, E. coli presents most often in immunocompromised or elderly individuals as sudden-onset meningitis or septicemia and has an annual mortality rate averaging 25% of all diagnosed case [8].

Material and Method

Garlic Juice (GJ) extraction: Fresh garlic bulbs (Iraqi white-yellowish species) were purchased from the south of Iraq farm. 200 g garlic bulbs were blended in 100 ml sterile distilled water (SDW). The juicer was used to produce a fine garlic juice. The supernatant was collected after centrifugation at 3000 rpm for 30 min and directly filtered (0.4 mM pore size, Millipore). Finally, I obtained 50% w/v, and the extract was Freeze at -20C° for the future experiments.

Garlic oil (GO): The Garlic oil was purchased from a farm. Chemical composition has been determined by HPLC to ensure having the primary component that could possess the antibacterial effect, data not shown.

Microbial Strains: Three hundred clinical isolates were cultured (on blood agar and MacConkey) from clinical specimens of patients at the private internal medicine center (south of Iraq). The isolated bacteria were obtained from the urine of cancer patients that suffer systemic infections and also from pregnant women. The isolates were identified as E.coli would be selected for the present study. The total of E.coli (n=75) was chosen for the present study. Bacterial identification using API 20 E (BioMerieux company, marry Eliote, France) was used for identifying E.coli. Bacterial staining standard microbiology methods were used to examine the purity of the isolated strains. Finally, Nutrient agar slants were used to store the isolated bacteria at 8°C until further consideration.

The minimum inhibitory concentration (MIC): MIC method was employed to find out the minimum concentration of an anti-microbial agent able to successfully inhibit the observable growth of a microorganism for 24 h incubated at 37°C. MIC has been determined using the method developed by Caldwell and Danzer [10] with minor modification.

Disc Diffusion Method: Whatman No.1 filter paper discs (6 mm in diameter) was impregnated in Fresh GJ solutions, the paper dried and has been cut into discs. 50 l of sterile distilled water (SDW) was added to discs to serve as a control. Discs were placed on Molar Hinton plate completely streaked with the bacteria. The plates were kept for 24 h at 37°C. Next day the inhibition zones were measured. Positive control 10 g Nitrofuration (Nit) and erythromycin (Ery) where used as a positive and negative control for E.coli, respectively [11]. 0.5 McFarland standard suspension was used to adjust the concentration of the suspension that has been made of the isolated strains.

The suspension was cultured on Mueller Hinton agar (Sigma Aldrich). The agar was incubated for 15 min at 37 °C to ensure that the agar will absorb the bacterial suspension. Finally, the disks containing the Amp, CF, FDG, and GO were placed on the agar using sterile forceps. A total of 5 replicates have been used in my study. After incubation for1 days at 37 °C, the plates were observed, and the diameters of the zones of inhibitions were measured using a ruler, table (1).

Protein Synthesis Assays: 150 ml liquid growth cultured aerobically at 37°C with the presence of unlabeled-DL-Leucine, then, the level of bacterial protein synthesis was detected via adding 10 μCi of L-(5.5-3H) leucine (Sigma Aldrich) to the bacteria. The 150 ml divided into three cylinders, 50ml had supplemented with 10ml GJ (1:5) dilution, the other supplemented with 10ml GO (1:5) dilution while the third left as a control. 2ml of the growth has been taken from each of the 3 cylinders divided into two tubes placed on ice, and each of them was received 0.1 ml of 5% perchloric acid. After 2 hours, all the samples were provided with 5ml of 5% cold perchloric acid and incubated on ice for half an hour. The liquid growth then filtered by 0.4 μm filter. Radioactive counter was used to measure the activity.

Statistical Analysis: SPSS software version 18.0 (SPSS Inc. Chicago, IL) was used to determine the significant correlation of the data. One-way ANOVA was employed to detect the correlation between the parameters, while the factorial experiment was performed to evaluate the relationship between the Garlic extracts and the bacterial growth with or without the antibiotics that have been used in my study. Finally, two-way ANOVA was used to determine the significant interaction as $P \geq 0.05$ was identified as a significant correlation.

Results and Discussion

Clinical Strains: From all the clinical samples that have been tested in the study, I isolated many strains. However, I chose 75 E.coli to do the further experiment.

Determine the Garlic derivatives of anti E.coli activities using discs diffusion methods: By measuring the diameter of the antibiotics, NIT was reflected resistant when the ZOI exactly ≤14, intermediate when it 15-16 and sensitive if it 17mm according to the manufacture instructions. Finally, Ery 15 μg was measured resistant when the ZOI is ≤13, intermediate if it 14-16 and sensitive when it 18mm.

The results that shows that NIT is highly toxic to E.coli which is scientifically expected according to [11]. While most of the E.coli strains were resistant to Ery and this results come a long previous data reported by [12]. GJ has shown an interesting result by successfully inhibiting 40 strain out of 75. The results comes along the data that suggested GJ can be toxic to variety of bacteria including MRSA [13]. Unfortunately, GO has shown toxicity to only 10 strains which is unexpected, since GO has reported to have high anti-bacterial activities [14].

My hypothesis stated that the garlic derivatives would post the antibiotic activity; therefore,

I used a combination of the antibiotics with either the GJ or GO. As I expected, the GJ significantly enhance the NIT activity and reduce the number of resistant and intermediate bacteria producing more sensitive bacteria. GO had enhanced the NIT activity. However, the difference did not reach essential effect. The combination of Ery and GJ have significantly increased the sensitivity of the bacteria; However, I am not exactly sure whether the reduction was due to the combination or it is solely from the Garlic derivatives (table 1). Therefore, further investigation is needed for exploring Garlic products effects. My project aims to eliminate the infecting organisms or to prevent the onset of E.coli diseases. The results explain that the GO and GJ exhibit selective toxicity as it demonstrates more significant toxicity to the infecting pathogens than to the host. The next step in the future research will focus on testing the antibiotics and the garlic derivatives on cell lines and animals.

Table 1: Test the antibiotics and the Garlic derivatives ability to reduce E.coli growth on the agar plates

Antibiotics	Isolated E. coli strain in two replicates	Sensitivity test determined by ZOI		
		Resistant N%	Intermediate N%	Sensitive N%
NIT	75	5	0	70
GJ	75	16	19	40
GO	75	50	15	10

Conted…

NIT+GJ	75	1	2	72
NIT+GO	75	1	3	71
Ery	75	67	3	5
Ery+ GJ	75	15	4	56
Ery+GO	75	40	13	22

Protein Synthesis Assays: A significant negative effect has been detected when measuring the bacterial growth that has supplemented with GJ as compared to the control group, p=0.02 (figure 1). The GO protein synthesis showed decreased in the protein synthesis, but the results did not reach a significant level, p=0.08 (figure 2). This data suggests that the GJ as it fresh could have a more anti-microbial effect than GO. Another possible explanation is that the GJ could have components that are a potent anti-microbial structure such as allicin in higher concentration than GO. This could be due to the oil extracting mechanism that might be harsh and caused unintentionally distraction and loss in the amount of allicin.

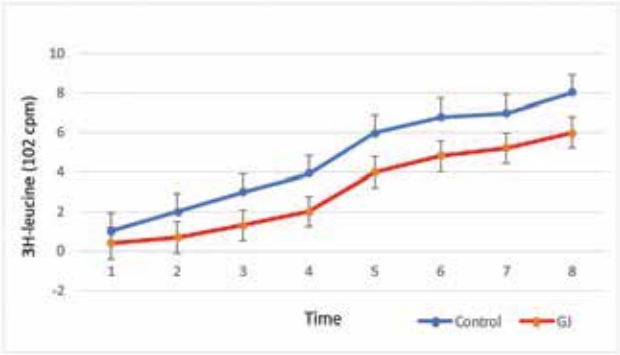

Figure 1: GJ has significantly reduced bacterial protein synthesis

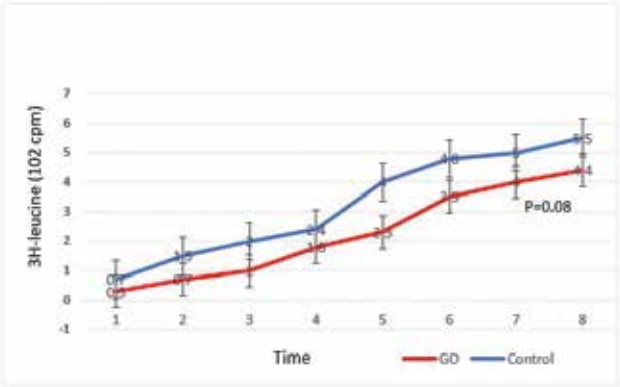

Figure 2: GO has the ability to reduce bacterial protein synthesis

Future Study: I recommend to test GJ and GO with antibiotics to treat resistant bacteria such as Neisseria Gonorrhea and MRSA.

Table 2: List of abbreviations

Abbreviation	Full Name
E. coli	Escherichia coli
SDW	sterile distilled water
GJ	Garlic Juice
GO	Garlic oil
Nit	Nitrofuration
Ery	erythromycin
ZOI	Zone of inhibition

Ethical Clearance: The study is a part of regular university of Sumer observation.

Conflict of Interest: The author has no conflict of interest.

Source of Funding: The author declared that the self fund has been used for this work.

REFERENCES

1. Lawson LD. Garlic: A Review of Its Medicinal Effects and Indicated Active Compounds. In: *Phytomedicines of Europe*. American Chemical Society, 1998, pp 176–209.
2. Bayan L, Koulivand PH, Gorji A. Garlic: a review of potential therapeutic effects. *Avicenna J Phytomedicine* 2014; **4**: 1–14.
3. Adetumbi M, Javor GT, Lau BH. Allium sativum (garlic) inhibits lipid synthesis by Candida albicans. *Antimicrob Agents Chemother* 1986; **30**: 499–501.
4. Cavallito CJ, Bailey JH. Allicin, the Antibacterial Principle of Allium sativum. I. Isolation, Physical Properties and Antibacterial Action. *J Am Chem Soc* 1944; **66**: 1950–1951.
5. Amonkar SV, Banerji A. Isolation and Characterization of Larvicidal Principle of Garlic. *Science* 1971; **174**: 1343.
6. Hughes BG, Lawson LD. Antimicrobial effects of Allium sativum L. (garlic), Allium ampeloprasum

L. (elephant garlic), and Allium cepa L. (onion), garlic compounds and commercial garlic supplement products. *Phytother Res* 1991; **5**: 154–158.

7. Kaper JB, Nataro JP, Mobley HLT. Pathogenic Escherichia coli. *Nat Rev Microbiol* 2004; **2**: 123.
8. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C *et al.* Food-related illness and death in the United States. *Emerg Infect Dis* 1999; **5**: 607–625.
9. Green JH, Wells JG, Greene KD, Barrett TJ, Bibb WF, Banatvala N *et al.* The United States National Prospective Hemolytic Uremic Syndrome Study: Microbiologic, Serologic, Clinical, and Epidemiologic Findings. *J Infect Dis* 2001; **183**: 1063–1070.
10. Caldwell DR, Danzer CJ. Effects of allyl sulfides on the growth of predominant gut anaerobes. *Curr Microbiol* 1988; **16**: 237–241.
11. Kibret M, Abera B. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. *Afr Health Sci* 2011; **11 Suppl 1**: S40–S45.
12. Arthur M, Courvalin P. Contribution of two different mechanisms to erythromycin resistance in Escherichia coli. *Antimicrob Agents Chemother* 1986; **30**: 694–700.
13. Li G, Ma X, Deng L, Zhao X, Wei Y, Gao Z *et al.* Fresh Garlic Extract Enhances the Antimicrobial Activities of Antibiotics on Resistant Strains in Vitro. *Jundishapur J Microbiol* 2015; **8**: e14814–e14814.
14. Ross ZM, O'Gara EA, Hill DJ, Sleightholme HV, Maslin DJ. Antimicrobial Properties of Garlic Oil against Human Enteric Bacteria: Evaluation of Methodologies and Comparisons with Garlic Oil Sulfides and Garlic Powder. *Appl Environ Microbiol* 2001; **67**: 475.