

**Evaluation of Antimicrobial Activity of Garlic (*Allium sativum*)
Against *E. coli* O₁₅₇:H₇**

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Abstract. Garlic extract with different concentrations (0.5, 1.0, 3.0 and 5.0 %) were tested for inhibitory activity against *E. coli* O₁₅₇:H₇ in MacConkey sorbitol agar (MSA). The reduction rates were 58, 100, 100 and 100%, respectively. Aqueous extract of garlic were tested for their inhibitory activity against *E. coli* O₁₅₇:H₇. The Minimal Inhibitory Concentration (MIC) of garlic extract against *E. coli* O₁₅₇:H₇ was 1.56% (w/v), while the Minimal Lethal Concentration (MLC) was 3.12% (w/v). Garlic extract concentrations which had the best inhibitory effects against *E. coli* O₁₅₇:H₇ in the laboratory medium and in-vitro study, should be chosen and tested for the food model study. The obtained results showed that garlic extract 3% has the highest inhibitory effect against *E. coli* O₁₅₇:H₇ at the 3rd day of storage with reduction rate of 100%.

Key words: Garlic, *E. coli*, inhibitory concentration, lethal concentration.

Introduction

Man has been using natural products of animals, plants and microbial sources for thousands of years either in the pure forms or crude extracts (Parekh and Chanda, 2007). Bioactive compounds from these diverse sources have been isolated and characterized worldwide. Systematic screening of plant materials represent an all important effort to find some new bioactive compounds with the needed therapeutic potential to fight against pathogenic microorganisms, particularly with respect to those that are hospital based. The elucidation of the chemical structures of some of these compounds had led to the synthesis and production of more potent and safer drugs. However, within the last few decades, microbial resistance has emerged for most of the available agents, thus necessitating the search for newer drugs (Bhattacharjee *et al.*, 2005). The increasing reliance on drugs from natural sources has led to the extraction and development of several drugs and chemotherapeutic agents from traditional herbs which are present in abundance in the tropics (Falodun *et al.*, 2006).

Garlic (*Allium sativum* Linn.) is one of those plants that was seriously investigated over the years. It has been used for centuries to fight infections (Onyeagba *et al.*, 2006). The early Egyptians used it to treat diarrhoea, the ancient Greeks used it to treat intestinal and extraintestinal diseases, while the ancient Japanese and Chinese used it to treat headache, flu, sore throat and fever. In Africa, particularly in Nigeria, it is used to treat abdominal discomfort, diarrhoea, otitis media and respiratory tract infections (Ankri and Mirelman, 1999; Jaber and Al-Mossawi, 2007). The phytochemical constituents of garlic have been established in previous studies (Farbman, *et al.*, 1993; Cavallito and Bailey, 1994; Ankri and Mirelman, 1999; Prados-Rosales *et al.*, 2003). The antimicrobial properties of garlic were first described by Pasteur in 1958, and since then, research had demonstrated its effectiveness against bacteria, protozoa, fungi and some viruses (Jaber and Al-Mossawi, 2007). Previous studies have also indicated that garlic has anti-neoplastic, cardiovascular, immuno-stimulatory and hypoglycemic properties (Sato and Miyata, 1999).

Garlic is a potent inhibitor of food pathogens. Foods contaminated with pathogens pose a potential danger to consumer health. Use of garlic would increase the shelf-life and decrease the possibilities of food poisoning and spoilage in processed foods. The Minimum inhibitory concentration (MIC) of garlic for *E. coli*, *Sal. typhi*, *Staph. aureus* and *L. monocytogenes* was 3.95, 7.0, 5.0 and 8.8%, respectively. The maximum inhibitory effect of garlic was observed against *E. coli* and the minimum against *L. monocytogenes*. In the case of *E. coli* and *Staph. aureus*, inhibition occurred rapidly. Up to the 5% level of garlic, there was an almost 80% inhibition and after that, very gradual inhibition was observed. (Kumar and Berwal, 1998).

It has consistently demonstrated the effectiveness of garlic against the nosocomial *S. aureus*, *E. coli*, *S. pneumoniae* and *P. aeruginosa* that frequently display above average resistance to many antimicrobial agents. If well processed, garlic preparations can be used to treat nosocomial infections caused by susceptible

bacteria. The ability of garlic to inhibit the growth of both gram-positive and gram-negative bacteria shows that it has a broad spectrum of activity and can be used for formulation of newer broad spectrum antibacterial substances (Abubakar, 2009).

The antimicrobial and synergistic effects of the plants, *Allium sativum* and *Gongronema latifolium* on *Escherichia coli* and *Staphylococcus aureus* were investigated. It is concluded that synergism associated with the combination of medicinal plants is doubtful. However, the synergistic or additive effect between garlic and conventional drugs to some strains of bacteria which are resistant to some conventional drugs, gives hope of fighting drug resistance (Eja, *et al.* 2011).

The aim of the present study is to Evaluation of antimicrobial activity of garlic (*Allium sativum*) against *E. coli* O₁₅₇:H₇

Materials and Methods:

Preparation of garlic extract: Garlic extract was prepared according to the methods described by Zahira and Al-Delaimy(1982) and Kumar and Berwal, (1998). Briefly, the garlic bulbs were cleaned with tap water and detergent (0.2 % mercuric chloride) for 2 min to remove any adhering soil on their surfaces followed by five to six washings with dist. water. 100 g of garlic were taken after removal of their outer skin surfaces and cut into small pieces by sterile scalpel. The small pieces were blended with 100 ml sterile dist. water using sterile warring blender for 5 min at medium speed. The macerates were filtered using sterile funnel and Whatman filter paper. The filtered extract was used for studies within 8 h of extract preparation. Two-fold serial dilutions were prepared from the extract previously prepared, i.e. 50.0, 25.0, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 % (w/v).

Bacterial strain: *E. coli* O₁₅₇:H₇ strain was isolated from beef burger sample and identified by PCR assay. A fresh culture was prepared by inoculating 10 ml of Tryptic Soy Broth (TSB) with a loopful of the stock culture and incubating the inoculated tube at 37°C for 18-20h.

In vitro methods:

Agar diffusion method (Parish and Davidson, 1993):

Petri dishes were prepared to contain a non-selective medium, Tryptic Soy Agar (TSA) at a depth of approximately 4mm. The medium was surface inoculated with a suspension containing approximately 1×10^7 cfu/ml of *E. coli* O₁₅₇:H₇, which were prepared in TSB culture after overnight incubation and adjusted to such previous initial inoculum. Known concentrations (100 µl) of each tested garlic extract were added by using sterile automatic micropipette to wells cut in the agar plate with a sterile glass tube (6 mm diameter). Negative control was prepared by placing sterile water in a well. Plates were incubated at 37°C for 24h. Following incubation; plates were examined for zones of inhibition that measured by using a vernier caliper. The evaluation of inhibitory properties was carried out in duplicate and the results were expressed as average values of inhibition halo. Minimum Inhibitory Concentration MIC for the tested extracts was predicted from the linear regression analysis of the relationship between the squared radii of the zone of inhibited growth versus logarithmic values of the concentrations.

Broth dilution method (Cosentino *et al.*, 1999 and Elgayyar *et al.*, 2001):

All tested extracts were performed in Brain Heart Infusion Broth (BHIB) supplemented with Tween 80 detergent at a final concentration of 0.5% for emulsification of essential oils in aqueous media. Serial doubling dilutions of each extracts were performed as previously mentioned. Overnight broth culture of *E. coli* O₁₅₇:H₇ were prepared in TSB and adjusted so that the final concentration of each tube following incubation was approximately 6×10^5 CFU/ml. The concentration of initial inoculums was confirmed using viable counts on TSA plates. A control tube should be prepared. The tubes were incubated at 37°C for 24h and minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) were determined. MIC was determined presumptively as the first tube that did not produce turbidity. MIC was defined as the lowest concentration of an antimicrobial that prevents growth of a microorganism after a specified incubation period [i.e., the lowest concentration at which no growth occurs (absence of turbidity) in a nutrient medium following incubation]. To confirm MIC and establish MLC, 0.1 of broth was removed from each tube and surface plated onto TSA. After aerobic incubation at 37 °C overnight, the number surviving *E. coli* O₁₅₇:H₇ was determined. The MIC was the lowest concentration which resulted in a significant decrease in inoculums viability (> 90 %) while, the MLC was defined as the lowest concentration that killed > 99.9 % of *E. coli* O₁₅₇:H₇ within 72h (MLC culture was incubated at 37°C for 24, 48, and 72h). Survival was determined by plating an aliquot from each tube into TSA plates incubated for 48 h at 37°C and recorded as (+) for growth or (-) for no growth.

In vivo method (Food model):

Spices and spice extracts which had the best inhibitory effects against *E. coli* O₁₅₇:H₇ in the laboratory medium and *in vitro* study, should be chosen and tested for the food model study.

Evaluation of garlic extracts in minced beef (Ceylan *et al.*, 1998):

500 g of fresh meat was purchased from a local butcher. The samples were minced and divided into five equal portions each of 100 g in sterile beakers. The 1st and 2nd beakers were received 1 & 3% (w/w) garlic, while the 3rd and 4th beakers received 1.56 and 3.12% (v/v) aqueous garlic extract. Whereas, the five portion was considered as control sample which had no garlic. *E. coli* O₁₅₇:H₇ was added to these mixtures to obtain 3×10^5 CFU/g initial inoculum level. Both the bacterial inoculums and garlic extracts were distributed in the minced meat by stomacher for 2 min. All beakers were covered with aluminum foil and refrigerated at 3°C and examined at the 1st, 2nd, 3rd and 7th days to evaluate the viable cell counts of *E. coli* O₁₅₇:H₇.

Evaluation of *E. coli* O157:H7 in the inoculated samples:

25g of the inoculated ground beef sample were transferred into sterile 250-stomacher bag together with 225 ml of sterile 0.1-peptone water. The sample was thoroughly homogenized by using a stomacher for 2 min. Serial dilutions of the homogenate were prepared by using 0.1-peptone water as diluents. 0.1 ml portions of three consecutive dilutions were spread-plated on MacConkey sorbitol agar (MSA) medium. The plates were incubated at 37°C for 24h.

Results

The obtained results were recorded in Tables (1), (2) and (3).

Table (1). Reduction rates of *E. coli* O₁₅₇:H₇ on manitol salt agar containing different concentrations of garlic

Garlic concentration	0.5 %	*R. R. (%)	1 %	R. R. (%)	3 %	R. R. (%)	5 %	R. R. (%)
Control	50x10 ⁵	0.0	50x10 ⁵	0.0	50x10 ⁵	0.0	50x10 ⁵	0.0
<i>E. coli</i> counts	21x10 ⁵	58	**NG	100	NG	100	NG	100

*Reduction rate

**No growth

Table (2). Minimal inhibitory and lethal concentrations of garlic extract on *E. coli* O₁₅₇:H₇

Antimicrobial activity of garlic extract	<i>E. coli</i> O ₁₅₇ :H ₇	
	*MIC	**MLC
Manitol Salt Agar	1.56 % (w/v)	3.12 % (w/v)
Agar diffusion	2.51 % (w/v)	-

*Minimal Inhibitory Concentration

**Minimal Lethal Concentration

Table (3). Reduction rates of different concentration of garlic extract on *E. coli* O₁₅₇:H₇ inoculated into minced meat

		After day 1	After days 2	After days 3	After 7 days
Control	mean	3X10 ⁵	2x10 ⁵	2x10 ⁵	1x10 ⁵
	R. R. (%)	0.0	0.0	0.0	0.0
Garlic 1 %	mean	3X10 ³	7x10 ²	10.0	10.0
	R. R. (%)	99.0	99.5	99.9	99.9
Garlic 3 %	mean	1X10 ³	1X10 ²	0.0	0.0
	R. R. (%)	99.6	99.9	100.0	100.0
Garlic 1.56 % (w/v)	mean	2X10 ⁴	8X10 ²	10.00	10.00
	R. R. (%)	99.3	99.6	99.9	99.9
Garlic 3.12 % (w/v)	mean	8X10 ³	3X10 ²	10	0.0
	R. R. (%)	97.3	99.8	99.9	100

Discussion

Garlic (*Allium sativum*) in the family *Liliaceae* is a perennial bulb-forming plant. It is known world-wide, and for several centuries, it has been used for dietary and medicinal purposes. Antimicrobial activity of garlic and its extract has been recognized for many years in all parts of the world. Scientific studies made on garlic in 20th century revealed that it was effective against a wide variety of microbial pathogens (Ross *et al.*, 2001). Garlic contains 0.3–0.5% allicin, an antimicrobial component (Shelef 1983). According to Zaika *et al.* (1983), the Gram- positive

bacteria are generally more sensitive to allicin than Gram-negative bacteria. Further studies have confirmed also that garlic and its extract has antimicrobial properties (Reuter *et al.*, 1996; Kumar and Berwal, 1998; Ross *et al.*, 2001; Martin and Ernst, 2003; Eja *et al.*, 2007; Eja *et al.*, 2011).

Effect of garlic on *E. coli* O₁₅₇:H₇ on MSA showed high antagonistic effect (strong inhibition), inhibiting the growth of *E. coli* O₁₅₇:H₇ totally at 1 %, while the control sample had 50×10^5 cfu/ml (Table 1). These findings were in harmony with those reported by Ceylan *et al.*, (1998). As well as, Abdou *et al.* (1972) concluded that 5–10% fresh garlic was sufficient to inhibit the growth of *E. coli*, *Shigella dysenteriae*, *Sal. typhosa* and *Staph. aureus* completely.

Current methods used to evaluate the efficacy of food antimicrobial may be divided into *in vitro* and *in vivo*. *In vitro* methods provide only preliminary information to determine the potential usefulness of the test compound in the food, whereas, application methods, in which an antimicrobial was applied directly to a food product to determine its effect on an inoculated microorganism (Parish and Davidson, 1993).

The obtained results given in Table (2) indicated that calculated MIC for aqueous garlic extract was 2.51.

In vivo method (Food model), the antimicrobial effect of garlic extract on the growth of *E. coli* O₁₅₇:H₇ in minced meat was carried out. The garlic extract proved to have a good inhibitory effect against *E. coli* O₁₅₇:H₇ in previous preliminary *in vitro* study which include: 1 and 3 % of garlic, 1.56 (MIC) and 3.12 (MLC) % (w/v) of aqueous garlic extract. Higher MIC (3.95 %) of garlic for *E. coli* rather than the obtained findings was recorded by Kumar and Berwal, (1998).

From the results outlined in Tables (3) it could be observed that garlic (3%) had the highest inhibitory effect on the growth of *E. coli* O₁₅₇:H₇ in minced meat stored at 3°C for 7 days with reduction rate of 100 % at the 3rd day of storage. Greater reductions were observed after 7 days of refrigerated storage, rather than immediately after application. Other researchers have noted that lower temperature and prolonged storage may enhance the inhibitory activity of plant extracts (Beuchat *et al.*, 1994 and Hao *et al.*, 1998).

The overall observation of the results of the food system showed that there is a partial decrease in the antimicrobial activity of garlic in contrast to their antimicrobial inhibitory effect in culture medium. Many factors in foods could be responsible for the reduction of antimicrobial activity of garlic extract while applied on different types of food. This observation was recorded by many investigators such as Ismaiel and Pierson, (1990) and Stechini *et al.* (1993) who reported that antimicrobial activity of spices and oils diminished in food as a result of solubility of the antimicrobial agents into the food's lipid fraction.

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تقييم نشاط الثوم كمضاد للميكروبات ضد *E. coli* O₁₅₇:H₇

محمد س. العريني

كلية الصيالة ، جامعة القصيم ، صندوق بريد ٦٨٠٠ بريدة ٥١٤٥٢ ، السعودية

قدم للنشر في ٢٠١١/٢/١٧ م ؛ وقبل للنشر في ٢٠١١/٦/٢٢ م

ملخص البحث. تم اختبار تركيزات مختلفة (٥.٠، ٣.٠، ١.٠، ٠.٥)٪ من مستخلص الثوم كمثبط لنشاط *E. coli* O₁₅₇:H₇ في آجار ماكونكي والسريتول. (MSA). وكانت نسب الخفض ٥٨ ، ١٠٠ ، ١٠٠ و ١٠٠ ٪ ، على التوالي. تم اختبار المستخلص المائي للثوم كمثبط لنشاط (*E. coli* O₁₅₇:H₇) ووجد أن التركيز الأدنى المثبط (MIC) من مستخلص الثوم هو ١.٥٦ ٪ (وزن/حجم) بينما التركيز الأدنى المميت (MLC) هو ٣.١٢ ٪ (وزن/حجم).

إن تركيزات مستخلص الثوم التي لديها أفضل الآثار المثبطة ضد *E. coli* O₁₅₇:H₇ في المختبر يجب أن يتم اختبارها واختبارها لدراسة نموذج الغذاء. النتائج التي تم الحصول عليها من الدراسة الحالية أظهرت أن مستخلص الثوم (٣٪) لديها أعلى تأثير مثبط ضد *E. coli* O₁₅₇:H₇ في اليوم الثالث من التخزين وكان معدل التخفيض يساوي ١٠٠ ٪.

الكلمات المفتاحية: الثوم ، الكولا ي ، التركيز المثبط ، التركيز المميت.