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INHIBITORY EFFECT OF AQUEOUS GARLIC EXTRACT (*ALLIUM SATIVUM*) ON SOME TYPES OF ENTEROBACTERIACEAE *IN VITRO*

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ABSTRACT

An un conventional technique was developed for a preliminary screening of some pathogens on the basis of their sensitive to garlic (*Allium sativum*), it was found that aqueous garlic extract were possess a potent bacteriostatic principle against some species of Enterobacteriaceae. In agar medium, high inhibition zone in garlic were 40 mm for *Shigella dysenteriae*, 38 mm for *E.coli* and 36 mm for *Salmonella typhi*. Also results showed moderate significant effects against growth of *Enterobacter aerogenes, Klebsiella pneumonia, Proteus mirabilis* and *Serratia marsecense* it reached to 31mm, 30mm, 29 mm, 24 mm in diameter respectively, it inhibited the growth of all these species which were resistant to some commonly used antibiotics. Most of the tested species were resistant to ciprofloxacin and tetracycline, although sensitive to garlic.

KEYWORDS: Garlic extract, Antibacterial sensitivity, Enterobacteriaceae.

INTRODUCTION

Garlic (Allium sativum *Linn.*), which is commonly used in culinary practices and folk medicines, has been reported to possess a broad spectrum antibacterial activity (Wills 1956; Al-Delaimy & Ali 1970; Sharma *et al.* 1977; Mantis *et al.* 1978; Srivastava *et al.* 1982; Sato *et al.* 1990; Waqar *et al.* 1994; Kumar & Berwal 1998). The antibacterial studies have revealed varying magnitudes of its effects depending on the organism, growth medium and garlic preparation used (Al-Del- aimy & Ali 1970; De Wit *et al.* 1979; Srivastava *et al.* 1982). The principal antimicrobial component of garlic is allicin (diallyl disulphide and diallyl trisulphide) (Cavallito & Baily 1944). Intact garlic bulbs do not contain allicin in its active form, Garlic (Allium sativum *Linn.*), which is commonly used in culinary practices and

folk medicines, has been reported to possess a broad spectrum antibacterial activity (Wills 1956; Al-Delaimy & Ali 1970; Sharma *et al.* 1977; Mantis *et al.* 1978; Srivastava *et al.* 1982; Sato *et al.* 1990; Waqar *et al.* 1994; Kumar & Berwal 1998). Garlic (Allium sativum *Linn.*), which is commonly used in culinary practices and folk medicines, has been reported to possess a broad spectrum antibacterial activity (Wills 1956; Al-Delaimy & Ali 1970; Sharma *et al.* 1977; Mantis *et al.* 1978; Srivastava *et al.* 1982; Sato *et al.* 1990; Waqar *et al.* 1994; Kumar & Berwal 1998). Garlic (Allium sativum *Linn.*), which is commonly used in culinary practices and folk medicines, has been reported to possess a broad spectrum antibacterial activity (Wills 1956; Al-Delaimy & Ali 1970; Sharma *et al.* 1977; Mantis *et al.* 1978; Srivastava *et al.* 1982; Sato *et al.* 1990; Waqar *et al.* 1994; Kumar & Berwal 1998). Garlic (Allium sativum *Linn*.), which is commonly used in culinary practices and folk medicines, has been reported to possess a broad spectrum antibacterial activity (Wills 1956; Al-Delaimy & Ali 1970; Sharma *et al.* 1977; Mantis *et al.* 1978; Srivastava *et al.* 1982; Sato *et al.* 1990; Waqar *et al.* 1994; Kumar & Berwal 1998).

We live today renaissance modern towards medical plants, scientific researches were proven that much of these plants (vegetable extracts) containing therapeutic compound that can inhibit the growth of pathogens Garlic (*Allium sativum*) which is commonly used in culinary practices and folk medicines has been reported to posses a broad spectrum antibacterial activity (Kumar and Berwal, 1998). Garlic is more effective with least side effects as compared to commercial antibiotics; as a result, they are used as an alternative remedy for treatment of various infections (Tepe *et al.*, 2004). Out of the many medicinal plants, garlic has an antimicrobial property which protects the host from other pathogens highlighting the importance of search for natural antimicrobial drugs (Bajpai *et al.*, 2005; Wojdylo *et al.*, 2007).

OBJECTIVES OF STUDY

1. Collection garlic fruits from different localities and extraction the Aqueous Garlic Extract.
2. Preparation and identification of some pathogenic species of Enterobacteriaceae.
3. Susceptibility tested of pathogens by agar wells diffusion method in *vitro*.

2. METHODS

2.1 Plant Sample Collection

Garlic has been taking samples from different shops in the local markets in Al-najaf al-ashraf city, it was mash and put in bags dry clean in the refrigerator until use in microbial studies in the laboratory.

2.2 Samples of testing bacteria

It was obtained on a range of pathogenic bacteria including (*E.coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter aerogenes*, *Salmonella typhi*, *Shigella dysenteriae* and *serratia marsecense*), from clinical specimens in microbiology laboratory of AL-Sadr teaching hospital and others from laboratory microbiology graduate studies in the department of biology / Faculty of Science / University of Kufa.

2.3 Preparation of Aqueoue Garlic Extract

Blending 10 gm of the garlic bulbs with 10 ml of distill water and mixing in an electric mixer and used directly in experiments (Reuter, Kock and Lawson, 1996).

2.4 Standred McFarland Turbidity(0.5) tube

The tube of 0.5 McFarland standred was prepared by adding 0.5 ml of a 1.175% (wt /vol)of barium chloride dehydrate ($BaCl_2.2H_2O$) solution to 99.5 ml of 1% (vol /vol) of 1% sulfuric acid (H_2SO_4), the turbidity standred was solubled into test tube similar to those used to prepare the inoculums suspension, the McFarland standred tubes were sealed with parafilm to prevent evaporation and stored in the dark and cold place for six months, and examined each month to check turbidity using a spectrophotometer, this solution has absorbance about (0.08-0.1) at 650 nm wavelength (CLSI, 2010).

2.5 Study Antimicrobial activity of Aqueous Garlic Extract by Agar wells diffusion method

Aqueous Garlic Extract (AGE) was prepared in a sterile laminar air flow chamber. This extract was considred as the 100% concentration of the extract, each tested bacteria was inoculated into 10 ml brain broth for activation and incubated for 24 hours at 37 C^o, assayed bacterial growth with McFarland tube, the culture was then swabbed on Muller Hinton agar plates, agar well were prepared by a sterile cork borer at 5 mm diameter in the center of the plates, then add 0.2 ml of garlic extract in the well and leave the inoculated plates for a short time for diffusion the extract followed by incubation the plates in the incubater for 24 hours at 37 C^o. The experiments were carried out in triplicate. After growth the microbial inhibition

zones (halo diameter) around the well were measured and compared with antibiotic as control.

3. RESULTS AND DISCUSSION

3.1 Effect of Aqueous Garlic Extract on some types of Enterobacteriaceae

An un conventional technique was developed for a preliminary screening of some pathogens from Enterobacteriaceae on the basis of their sensitive to garlic (*Allium sativum*). The production of an inhibition zone in all tested of Enterobacteriaceae species indicated the strains were sensitive to garlic, a relative degree of sensitivity among the tested pathogens.

The Aqueous garlic extract of *Allium sativum* of some of local garlic fruit were evaluated against several pathogens from Enterobacteriaceae The results in the table (1) below showed that the aqueous garlic extract give a significant result against all tested bacteria. The maximum efficacy of garlic extract was on *Shigella dysenteriae* (figure 1) it reached to (40) mm in diameter although this species was low sensitive to tetracycline it reached to (7) mm inhibition zone, followed by *E.coli*, *Salmonella typhi* the inhibition zone diameter reached to (38 and 36) mm (figure 2, 3), although *E.coli* was resistant to rifampin antibiotic and *Salmonella typhi* was less sensitive to tetracycline it reached to (6) mm in diameter. The moderate inhibitory effect of aqueous garlic extract was found in *Enterobacter aerogenes* it reached to (31) mm in diameter (figure 4) followed by *Klebsiella pneumoniae* it give (30) mm in diameter (figure 5) although these two species were less sensitive to ciprofloxacin. The same is in the case for *Proteus mirabilis* (figure 6) and *Serratia marsecense* (figure 7) also they showed inhibitory effect reached to (29 and 24) mm respectively, the results indicate that a natural antimicrobial source (garlic extract) can be an alternative to some well known antibiotics.

Table 1: inhibition zone diameter (mm) of aqueous garlic extract against some Enterobacteriaceae species on Muller Hinton media.

No.	Organisms	Halo diameter (mm) of aqueous garlic extract	Halo diameter (mm) of Antibiotic (control)
1	*E.coli*	38	0
2	*Enterobacter aerogenes*	31	11
3	*Klebsiella pneumoniae*	30	13
4	*Proteus mirabilis*	29	6
5	*Shigella dysenteriae*	40	7
6	*Salmonella typhi*	36	6
7	*Serratia marsecense*	24	7

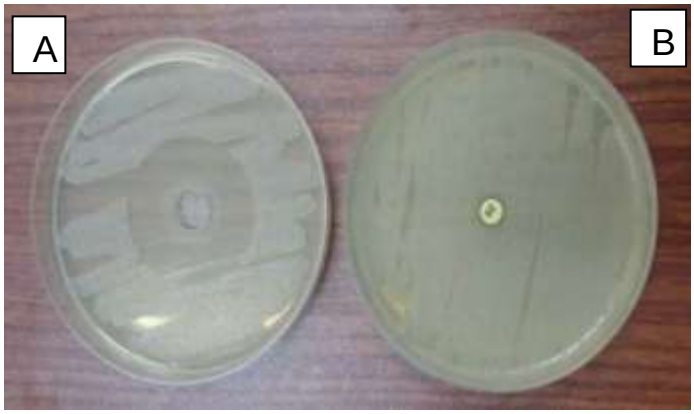

Figure (1)

A- Inhibition zones of aqueous garlic extract on *Shigella dysenteriae*.

B -Inhibition zones of Tetracycline on *Shigella dysenteriae* (control).

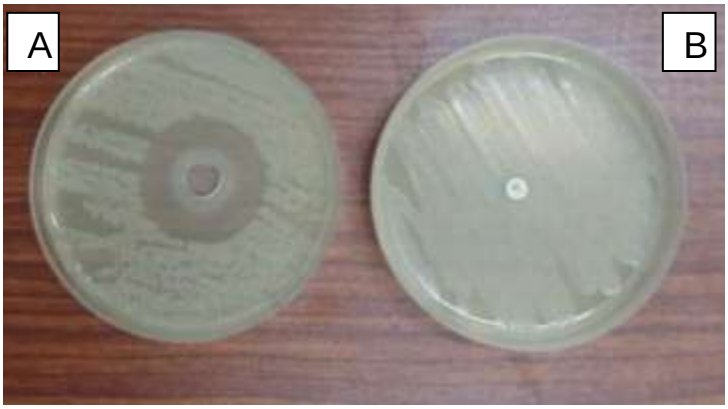

Figure (2)

A- Inhibition zones of aqueous garlic extract on *E.coli*

B -Inhibition zones of Rifampin on *E.coli* (control)

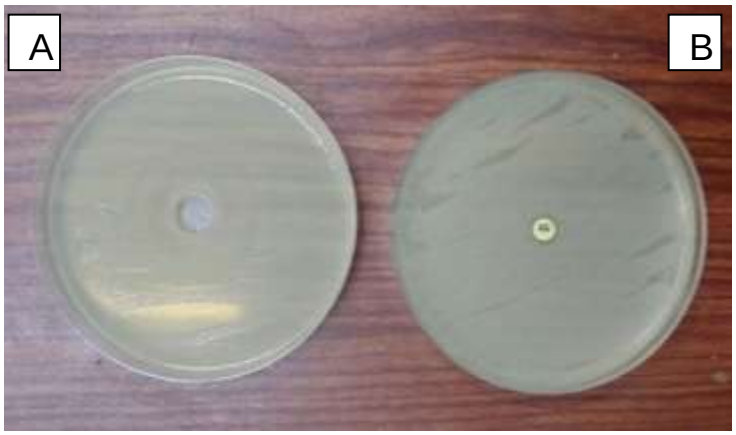

Figure (3)

A- Inhibition zones of aqueous garlic extract on *Salmonella typhi.*

B -Inhibition zones of Tetracycline on *Salmonella typhi.*

Figure (4)

A- Inhibition zones of aqueous garlic extract on *Enterobacter aerogenes.*

B -Inhibition zones of Ciprofloxacin on *Enterobacter aerogenes.*

Figure (5)

A- Inhibition zones of aqueous garlic extract on *Klebsiella pneumoniae.*

B -Inhibition zones of Ciprofloxacin on *Klebsiella pneumonia.*

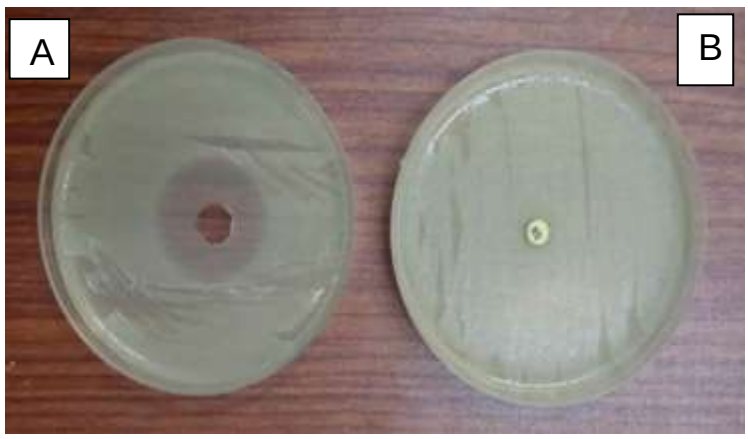

Figure (6)

A- Inhibition zones of aqueous garlic extract on *Proteus mirabilis.*

B -Inhibition zones of tetracycline on *Proteus mirabilis.*

Figure (7)

A- Inhibition zones of aqueous garlic extract on *Serratia marsecense.*

B -Inhibition zones of trimethoprime on *Serratia marsecense.*

Garlic extract inhibits the growth of Gram positive and Gram negative bacteria, such as *Staphylococcus, Streptococcus, Micrococcus, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Pseudomonas, Shigella, Salmonella, Proteus,* and *Helicobacter pylori* (Tsao and Yin, 2001).

Garlic was bacteriostatic, as was previously described by Srivastava *et al.*,(1982) in their studies on Gram negative bacteria.

The findings are similar to those of Kumar and Sharma (1982) were *E.coli* enterotoxin production was inhibited significantly by garlic extract Although literature on the effects of garlic on the growth of foodborne bacterial pathogens is available, they appear to be no earlier report on inhibitory effects of garlic on the production of enterotoxins by sporeformers.

Al-Delaimy and Ali (1970) reported that 4% (w/v) of fresh garlic in extract inhibited the growth of *E.coli, Shigella dysenteriae* and *S.typhi*.

Our observations confirm the long standing concept of using garlic in culinary practices as a means of securing safety and extending the shelf - life of food.

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