

Comparative in Vitro Activity of *Allium Sativum* (garlic) Aqueous Extract with Other Selected Antibiotics against *Brucella Melitensis*

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ABSTRACT

The aqueous *Allium sativum* crude extract was tested *in vitro* by using the two standard techniques, Zone-of-Inhibition (Kurbey and Bauer) and Turbidometric assays against some of the common *Brucella melitensis* clinical isolates in Mosul . It was found that the concentrated aqueous crude extract (1000 mg/ml D.W) of *A. sativum* had the most powerful inhibition activity against the dose 1×10^7 viable bacteria /ml compared with the rest concentrations of *A.sativum* used. These inhibitory activities of *A.sativum* crude extracts were compared with the standard antibiotic zones commonly used for the brucella bacteria therapy. It was found that these following concentrations 0.002, 0.03, 0.008, 0.07 and 0.7^{-6} mg/ml were the standards for the antibiotics Tetracycline, Ampicillin, Erythromycin, Streptomycin and Rifampicin respectively. The *in vivo* work is under estimation in our laboratory. However, such encouraging results suggest the alternative herbal therapy against Brucella species in future.

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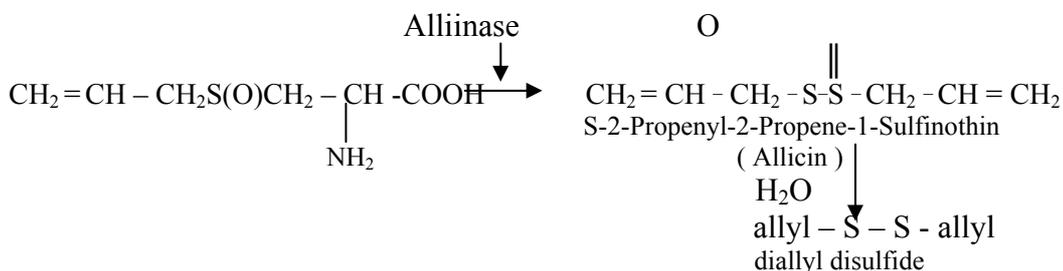
0.03 0.002 (/ 1000)
(/ $10^7 \times 1$)
/ 0.7^{-6} 0.07 0.008
in vivo

INTRODUCTION

1-Brucellosis: is a world problem of both public health and economic importance (Madkour et al., 1985). It is transmitted to human by ingestion of contaminated milk or from derivatives, especially cream and in many countries fresh cheese or possibly raw meat from infected animal, contact with animal or their products is a cause of infection in veterinarians, abattoir workers, farmers and who work with animals and their products, however, person to person spread is rare (Al-Sharbaaf et al., 1988; Young, 1989). The disease in man was reported early as 1938 (Dajani et al., 1989). In Iraq very few reports on Brucellosis among cattle, goats, sheep and camels are available.

Chemotherapy of Brucellosis has been some what disappointing, and disease respond irregularly and temporarily to ordinary regimes of antibiotics (Doll, 1977; Gotuzz et al., 1986). Therapeutic failures are probably due to the intracellular nature of the infection, since it was shown that intracellular bacteria are not readily destroyed by antibiotics (Weed et al., 1952; Gazapo et al., 1989). However, Brucellosis are now treated by Ampicillin, Rifampicin, Streptomycin, and Tetracycline (Young, 1995). This study was conducted to compare the *in vitro* activities of the *Allium sativum* extract with those of Ampicillin, Rifampicin, Streptomycin, Tetracycline and Erythromycin against *Brucella melitensis* isolates.

2-*Allium sativum*: Herbal plants belong to the family Liliaceae wide spread in many parts in the world. The English name of this plant, is garlic, part used; Bulbs, which contain a powerful bactericidal agent, allicin, which is formed by the reaction of *A. sativum* enzyme alliinase with the substrate alliin) (Koch et al., 1989), as seen in this formula:



Aim of this study: To investigate the inhibitory effect(s) *in vitro* of *A. sativum* crude aqueous extract and to compare this activity with the antibiotics commonly used for brucellosis therapy.

MATERIALS AND METHODS

1-Bacterial suspension: Inoculate *B. melitensis* on brucella agar plate and incubate for 24-48 hour at 37°C. The culture of Brucella bacteria was harvested and diluted with saline solution to give a suspension of about 10⁷ viable bacteria/ml, by using slide counting chamber (Hawksley, Gallenkamp/ U.K.).

2-Aqueous solution of *A. sativum*: The dried bulbs of *A. sativum* were ground and used as powder. Ten gram of this powder was put in distilled water for 24 hour and at room temperature. Shaking was done every now and then. The solution was then filtered and then dried by using Lyophilizer (Edward High Vacuum/U.K.) (Abbruzzese, 1987).

One milliliter of distilled water was added to each gram of *A. sativum* powder. An additional amounts of distilled water was used in order to prepare five dilutions as follows:

Mg/ml (powder of <i>A. sativum</i> /D.W.)	Dilutions* (Concentrations)
1000mg/ml	Concentrated
100mg/ml	10^{-1}
10mg/ml	10^{-2}
1mg/ml	10^{-3}
0.1mg/ml	10^{-4}
0.01mg/ml	10^{-5}

* All dilutions were sterilized by using membrane filter 0.22 μ m.

3-Test the inhibitory effect of aqueous solution of *A. sativum* and the antibiotic sensitivity: In this test Oxoid muller Hinton agar was used as ring diffusion around discs as a modified method of Kirby and Bauer (Michael and Aulton, 1988; Gerald et al., 1996).

4-Antibiotic disc: antibiotic disc (Oxoid LTD England) of 5 types was used for the sensitivity test and for comparison with activity of *A. sativum* extract.

5-Statistical analysis: F-test was used to evaluate the inhibitory effect at both levels ($p \leq 0.05-0.01$).

RESULTS AND DISCUSSION

The Zone-of-Inhibition assay revealed that the concentrated crude extract (1000 mg/ml) of *A. sativum* had the most powerful inhibition activity against the dose 1×10^7 viable bacteria/ml compared with the rest extracts (concentrations) with a significant effect on 0.01 and 0.05 levels as shown in figure (1,2).

However, the concentration (10 mg/ml) showed some higher inhibition than that of the (100 mg/ml). The rest serial concentrations showed gradual decrease in the inhibitory activities along with concentrations of *A. sativum* which explains that the higher concentration of the aqueous *A. sativum* the higher the inhibition occurs.

In Turbidometric assay which revealed that the concentrated crude extract of *A. sativum* had the most powerful inhibition activity to bacterial growth compared with the rest concentrations with asignificant effect on 0.01 and 0.05 levels as shown in figure (3).

Minimum inhibitory concentration of *A. sativum* crude extracts (MIC).

All results obtained from the sensitivity testing (Zone-of-Inhibition) with *A. sativum* crude extract showed real significant activity compared with the standard curve of the sensitive antibiotics which were used for treatment of Brucellosis (Table 1). This table explains the best minimum inhibitory concentration of *A. sativum* extract, also showed the lowest concentration of this extract at which there was no visible growth of brucella bacteria. Table (2) explains the relationship between the inhibitory effect of *A. sativum* and its concentrations on *B. melitensis* bacteria in comparison with some antibiotics.

It appeared from the results that the aqueous crude extract of *A. sativum* had an inhibitory effect to brucella bacteria at least in an *in vitro* studies done, (See Figs. 1, 2 and 3).

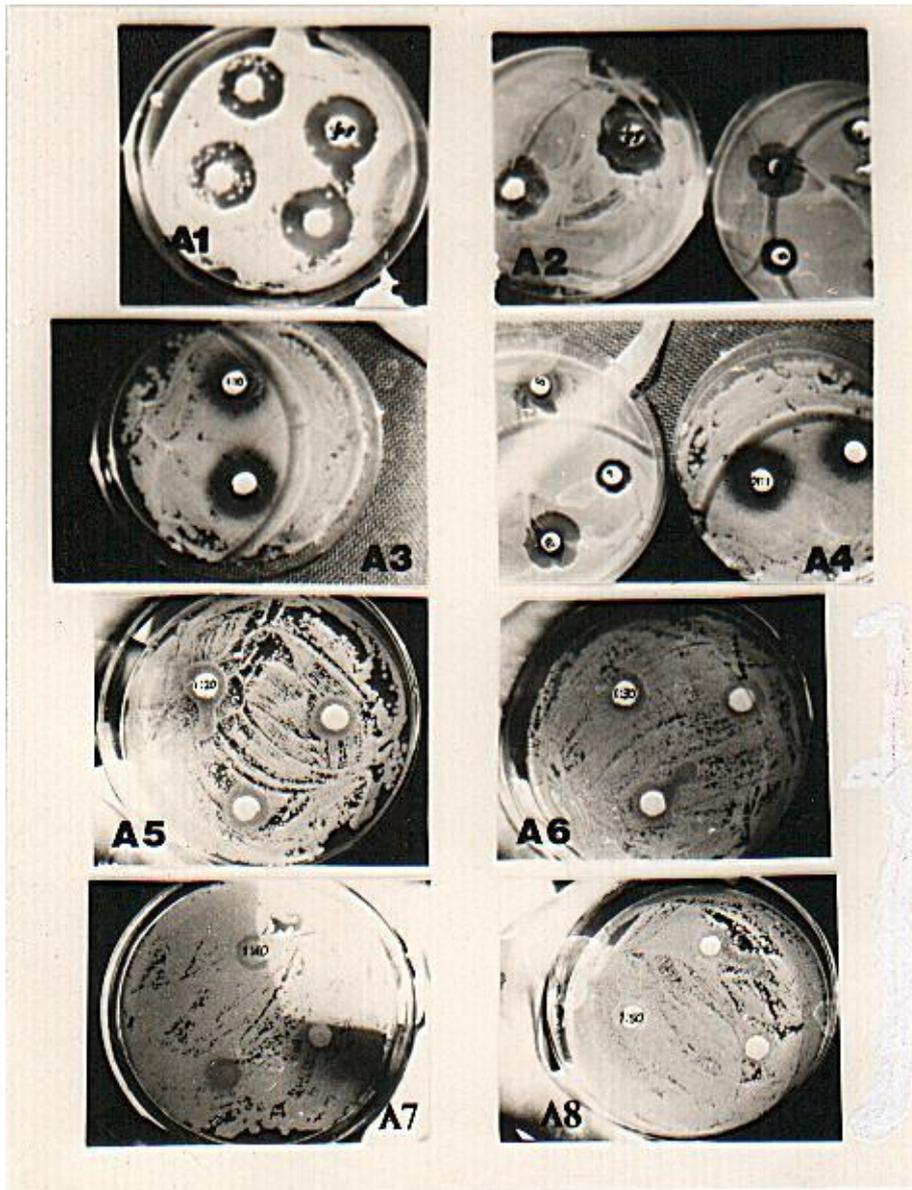


Fig.1: Anti *Brucella melitensis* activity of crude aqueous extract of *Allium sativum* by using the Zone Inhibition test.

A1= Inhibitory effect of (1000mg/ml D.W.)

A2=Inhibitory effect of (A1) compared with some sensitive antibiotics (Tetracyclin, Ampicillin, Streptomycin) to burcella bacteria.

A3= Inhibitory effect of (100mg/ml D.W.)

A4=Inhibitory effect of (A3) compared with some sensitive antibiotics (Tetracyclin, Ampicillin, Streptomycin).

A5= Inhibitory effect of (10mg/ml D.W.)

A6= Inhibitory effect of (1 mg/ml D.W.)

A7= Inhibitory effect of (0.1 mg/ml D.W.)

A8= Inhibitory effect of (0.01 mg/ml D.W.)

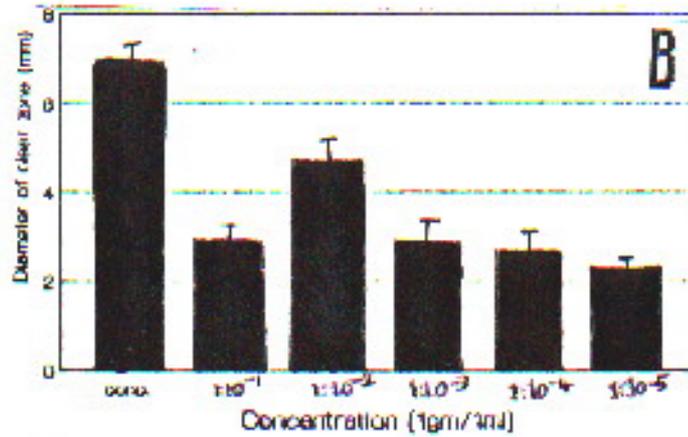


Fig.2: Anti *Brucella melitensis* activity of the crude aqueous extract of *Allium sativum*, using the Zone Inhibition test.

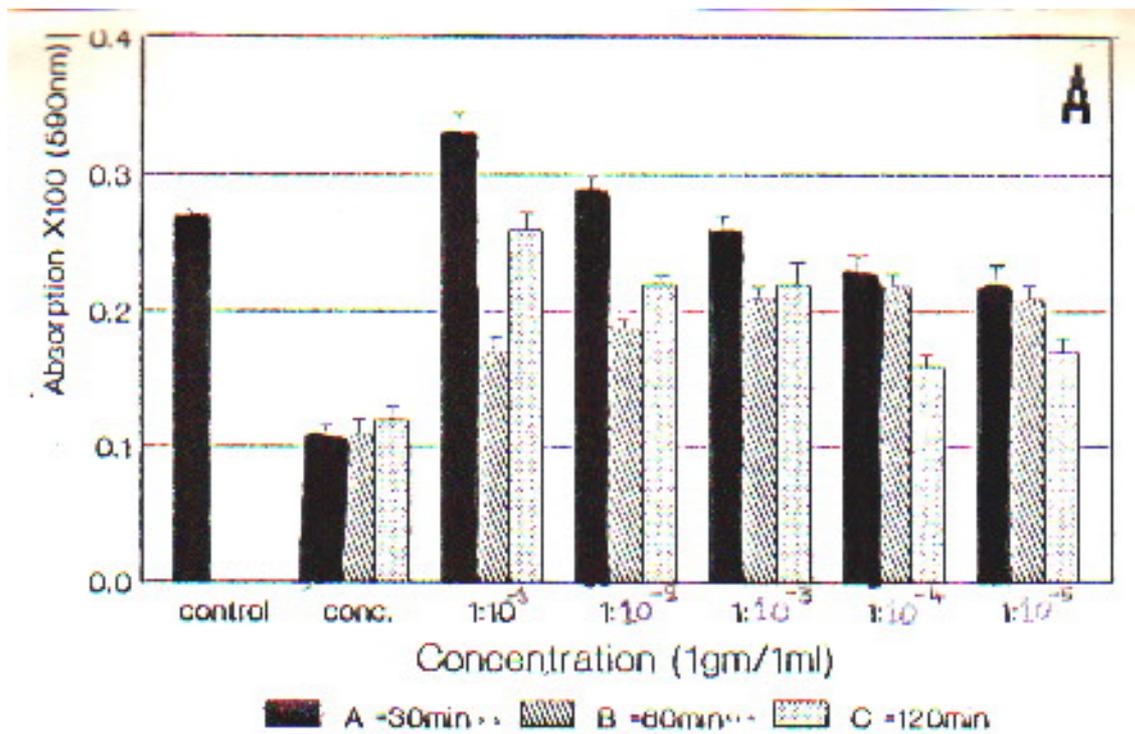


Fig.3: Anti *Brucella melitensis* activity of crude aqueous extract of *Allium sativum*, using the Turbidometric assay. One volume of the plant extract to one volume of 1×10^7 viable brucella bacteria /ml was used. (Note -using three time exposure).

Table 1: The Minimum Inhibitory Concentration (MIC) of *Allium sativum* crude aqueous extract to *Brucella melitensis* dose (1×10^7 bact. cell/ml) compared with the sensitive antibiotics, commonly used in medical therapy.

	MIC		
	Antibiotic mg/ml		<i>A.sativum</i> extract mg/ml
Tetracyclin	5.4^{-4}	=	0.1
Ampicillin	8.4^{-3}	=	0.1
Erythromycin	8.7^{-4}	=	0.1
Streptomycin	1.7^{-2}	=	0.1
Rifampicin	1^{-7}	=	1.0

Table 2: The relationship between the inhibitory effect of *Allium sativum* Crud aqueous extract to *Brucella melitensis* dose (1×10^7 bact. cell / ml) Compared with the sensitive antibiotics, commonly used in medical therapy.

Concentration Antibiotics Plant Extract	Tetracyclin e mg/ml	Ampicilli n mg/ml	Erythromyci n mg/ml	Streptomyci n mg/ml	Rifampici n mg/ml
	Concentrated 1000 mg/ml	0.002	0.03	0.008	0.07
100 mg/ml	0.0007	0.009	0.002	0.03	9.8^{-8}
10 mg/ml	0.0008	0.01	0.004	0.04	0.3^{-6}
1 mg/ml	0.0008	0.01	0.003	0.03	0.1^{-6}
0.1 mg/ml	0.0006	0.008	0.0009	0.02	0.3^{-6}
0.01 mg/ml	0.0006	0.009	0.0009	0.02	7.5^{-8}

The inhibitory effect of the extract might be due to the presence of one or more of the volatile oil (Weisberger and Pansky, 1957). Antimicrobials and sulphur containing components which present in this extract, as many others which have isolated and studied the antimicrobial activity from *A.sativum*. Kotb in 1985, for instance found that: Allistatian I, Allistatin II, garlicin, scordinin where the most active antimicrobial activities against fungus (Kotb, 1985). Several medicinal plants have been used in the world to treat infections. In India for example, over 30 Indian Folkloric medicinal plants have been tested against different bacterial isolates such as *Staphylococcus aureus*, *Streptococcus* species, *Escherishia coli*, *Proteus*, *Klebsiella* species and *Pseudomonas* species, Al-Delaimy (1988) in another study which confirmed the above antimicrobial activity against some gram negative bacteria such as *pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Salmonella typhimurium* in food born pathogenic bacteria (Al-Delaimy and Ali, 1988).

Many scientists had proved that this extract and its volalile oil contain a strong bacteriocidal agent, allyl thiosulfinic allyl ester (allicin) (Graham and Ernest, 1988; Sparnins et al., 1988).

Abbruzzese had proved in 1987, the effect of *A. sativum* extract has a strong inhibitory effect on 4 resistant strains of *Mycobacterium tuberculosis* (T.B) (Abbruzzese et al., 1987).

Interstingly Lutomski in 1988 have found that the aqueous garlic extract had the superior inhibitory activity against some different bacterial isolates compared with the most powerful commercial antibiotics used (Lutomski et al., 1988). This might be due to the ability of these compounds to diffuse into the tissue or it might be of the distribution better than the chemical compounds present in the some antibiotics and this effect become more evident in some bacteria especially on gram negative bacteria (Daiji, 1962).

All the above explain the economic use of such concentration of *A. sativum* as a chemical therapy in our country.

This study might suggest the possibility of using such medical plant as an alternative therapy against Brucellosis and also suggest further *in vivo* investigation.

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