

## In Vitro Antibacterial Activity of *Solanum Lycopersicum* Extract against some Pathogenic Bacteria

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### Abstract

The aqueous extract of tomato evaluated for activity against medically important bacteria *Escherichia coli*, *Klebsiella sp.* , *Pseudomonas sp* *Acinetobacter*, this research was screened the capability of the extract to inhibit the growth of some isolated bacteria. The in vitro antimicrobial activity was performed by agar well diffusion method. The aqueous tomato extract showed the maximum antimicrobial activity when diluted with honey than diluted with distal water . Sensitivity and resistance to extract and antibiotic varied from bacteria to another but *Solanum lycopersicum* proved active effects against all bacteria at pure extract without resistance with inhibition zone ranged(35-50) mm when we mixed *Solanum lycopersicum* with honey. The extract exhibited the higher activity until in diluted concentrations ,comparative by using the extract alone .

The use of tomato extract with known antimicrobial properties, can be of great significance in therapeutic treatments in vivo.

**Keywords:** tomato, *Solanum lycopersicum* ,aqueous extract, antibacterial activity, diluted with distal water &honey

### Introduction

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Selvamohan *et al.*2012). In this study, we investigated the antibacterial activity of aqueous extracts of *Solanum lycopersicum* against Gram negative pathogenic bacteria. To our knowledge, antibacterial activities of this plants against pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* (Unnisa *et al.*2012). Tomato is a red plant . It is spread throughout the world belongs to the plant family *Solanaceae* and genus *Solanum* (Sarah *et al.*2003). *Solanum lycopersicum* (tomato) is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components (Krishna *et al.*2013). used as juice, soup, puree, ketchup or paste (Dilis *et al.*2010). in terms of human health, tomato is believed to benefit the heart, among other organs. They contain the carotene lycopene, one of the most powerful natural antioxidants. In addition to its flavor properties, tomatoes are reported to possess numerous beneficial nutritional and bioactive components that may also benefit human health. These include the nutrients vitamin A, vitamin C, iron, and potassium (Frusciante *et al.*2007; Dorais *et al.*2008). Tomatoes are also an excellent source of free radical-scavenging bone-healthy vitamin K., vitamin B6, folate. In addition, tomatoes are a good source of heart-healthy magnesium, niacin, and vitamin E, vitamin B1, and phosphorus; muscle-building protein, and bone-healthy copper (Jacob *et al.*2010). Nonnutritive digestible and indigestible dietary fiber; the antioxidative compounds:  $\beta$ -carotene and lutein; (Frusciante *et al.*2007) and the cholesterol lowering and immune system enhancing glycoalkaloids tomatine and dehydrotomatine (Morrow *et al.*2004). Consumption of tomatoes, tomato products, and isolated bioactive tomato ingredients is reported to be associated with lowered risk of cancer (Friedman *et al.*2007) heart disease, diabetes (Bose *et al.*2007) and hypertension (Engelhard *et al.*2006). These considerations suggest that edible tomato contains antimicrobials which may have multiple benefits.

The major carotenoid in tomato is Lycopene

shows strong antioxidant activity. Lycopene, the red pigment of tomato, is a tetraterpene assembled from eight isoprene units composed entirely of carbon and hydrogen, containing

11 conjugated and two nonconjugated carbon-carbon

double bonds (Agarwal *et al.*2000)

### MATERIALS AND METHODS

Collection of plant & honey

Tomato & honey have been used in the present study and was collected from locally market at Baghdad , Iraq.

Processing of plant

The tomato collected was washed with water to remove the soil and dust particles , then surface sterilized with

70% alcohol and then rinsed with sterile water. it was dried in thoroughly sterile place, and grinding to form a fine powder and stored in airtight containers.

### **Bacterial strains**

The clinical isolated pathogens such as *Klebsiella pneumoniae*, *pseudomonas aeruginosa*, *Escherichia coli* and *Acinetobacter* were obtained from high studies Laboratory in collage of science, AL-Mustansiriyah university. these isolates were identified by the chemical test & the stock culture were kept in refrigerator 4°C on nutrient agar.

### **Bacterial suspension preparation**

After activated the isolates in brain heart infusion broth. the inoculated broth tubes were incubated at 37°C for 24 hours. until obtain of growth by observed the tubes. bacterial suspension containing bacteria of about  $10^6$  cell / ml was prepared with sterile physiological saline for further use.

**Antibiotic used and susceptibility testing** :Antibiogram of the isolates was ascertained on Muller Hinton Agar using disc diffusion method. Four antibiotics most commonly used for the treatment UTI were employed. The diameter of the zone of inhibition produced by each antibiotic disc was measured, recorded and the isolates were classified as resistant or sensitive based on (CLSI).

### **Conventional antibiotic disc assay**

In the other hand, antibiotic sensitivity test used to assay the sensitivity pattern of the test organisms in compares to the tomato extract. The antibiotics and concentrations impregnated to the disc arms are Amoxicillin (AMX) 30µg, Nitrofurantion (NIT) 300µg, levofloxacin (LF) 30 µg and Ampicillin (AMP) 10 µg. However the discs were picked with a sterile forceps and positioned at the centre of the seeded muller hinton agar plates

### **Plant extraction method**

The aqueous extract of plant were prepared by of plants was prepared by dissolving 10gm of fine powder in 100 ml of distilled water sterilized for 24 hours. The contents was filtered by Whatman No.1 filter paper for 10 mint. Then the extract was stored in sterilized bottles and They were kept under refrigerated condition unless they were used for the experiment to where the use. then Prepared the required concentrations (25,50,75,100)% by two method. first the mentioned Concentrations of extract prepared by take the appropriate amount of the filtrated and dissolved it in a certain volume of sterile distilled water, On the other hand, honey used to diluted the same concentrations.

### **COMBINATIONS OF Plant & honey**

After preparation the tomato extract. the combination, tomato : Honey was prepare; The extract was diluted with honey at 25:75, 50:50, 75:25, 100:100(pure honey), to obtain the concentration of 25% (v/v), 50%(v/v), 75 % (v/v) & 100% Respectively.

### **Assay of Antimicrobial activity using Agar well diffusion method:**

Petri plates containing 20ml nutrient agar, after solidification, were swabbed by ( $1.5 \times 10^8$  CFU/ml) of each bacterium and spreaded with sterile swabs. thus were left at room temperature for 15 minutes allowing the absorption of the inoculums into the agar. Wells or cups of 7 mm size were made with sterile cork borer into agar plates containing the bacterial inoculums. 50 µl volume of the plant extract was added into a well of inoculated plates. Sterilized distilled water was used as a negative control which was introduced into a well instead of tomato extract. Then, the plates incubated at 37 C, for overnight. The diameter of the zones of inhibition was measured with scale.

## **RESULTS**

Depending on microscopic and cultural examinations, the bacterial cultures isolates were confirmed the diagnosis. Bacterial identification characteristics of the *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Acinetobacter* and *Escherichia coli* were checked with API-20E system. The colonial and cell morphologies are shown in Table-1

**Table 1**  
**Colonial and cell morphology for clinical isolates of bacteria**

Bacteria species	Grams nature & morphology	capsule	motility	phonotype characteristics
<i>Klebsiella pneumoniae</i>	Gram negative large rods in short chains	+	hasn't flagella	Moist & mucoid raised creamy colonies.
<i>Escherichia coli</i>	Gram negative small single rods	-	has flagella	On EMB <i>E.coli</i> it will give a metallic green sheen.
<i>Acinetobacter</i>	Gram negative rods, some strains appear coccobacilli	+	hasn't flagella	On cultures Colonies are 1 to 2 mm, no pigmented, domed, and muciod, with smooth to pitted surfaces.
<i>Pseudomonas aeruginosa</i>	Gram negative small single rods	-	has flagella	Smooth and shiny colonies with green pigments

The antimicrobial activity of aqueous tomato extract Investigated by using agar well diffusion method against selected human pathogens such as *Klebsiella pneumoniae*,

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***Pseudomonas aeruginosa*, *Escherichia coli* and *Acinetobacter***

The results of aqueous tomato extract that diluted with D.W & honey, showed varied degree of antimicrobial activity against the pathogens .

There was significant variation in the antibacterial activities (DIZ values) of tomato aqueous extract . more activity was observed with honey diluted towards all four bacterial isolates than D.W diluted extract antibacterial activity used with diameter of inhibition zone (DIZ) values between 35mm and 50 mm of crude tomato extract that shown (Table – 2 and Figure-1)

**Table2 Antibacterial activity of extract diluted with D.W**

Organisms	Aqueous tomato extract diluted by distal water			
	Diameter of inhibition zone in mm			
	25%	50%	75%	100%
<i>Klebsiella pneumoniae</i>	-	-	-	40
<i>Escherichia coli</i>	-	-	-	50
<i>Acinetobacter</i>	-	-	-	35
<i>pseudomonas aeruginosa</i>	-	-	-	40

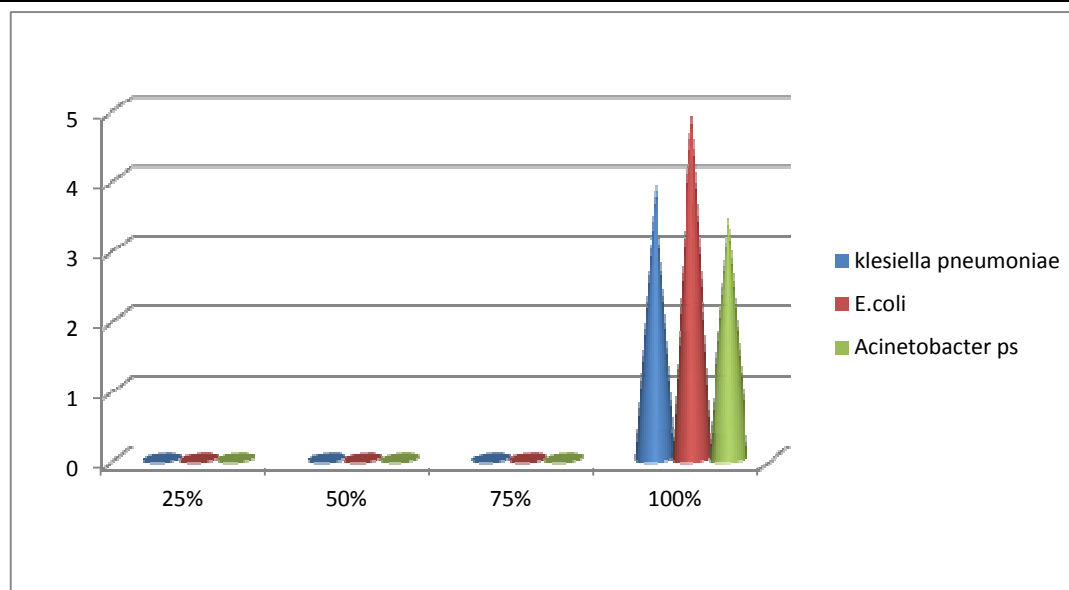


Figure 1 Diameter of inhibition zones of crude tomato extract & diluted with D.W on selected bacterial isolates.

Maximum inhibitory effect was At a concentration of 100% recorded for *E.coli* , 50mm; followed by *Klebsiella pneumoniae*, 40 mm; *Pseudomonas aeruginosa*, 40 mm; and *Acinetobacter sp* , 35 mm, Whereas no inhibition zone at concentrations 75%,50%,25% . The results antibacterial effect of extract that diluted with D.W lower than diluted with honey , the extract that diluted with honey more antibacterial effect against selected bacteria showed high activity compared with *E.coli*, *klebsella pneumoniae* , *Acinobacter* , *Pseudomonas aeruginosa*. The diameter of inhibition zone (DIZ) value was between 49 and 60 mm (table -3 &figure -2).maximum inhibitory effect was recorded for *E.coli* , 60mm ; followed by *klebsella pneumoniae* 55 mm ; *Pseudomonas aeruginosa*, 50mm and least on *Acinobacter sp*, 49mm.

At a concentration 75% , the DIZ values ranging between 50mm and 38mm. high effect was shown by *E.coli* , 50 mm; then for *klebsiella pneumoniae*, 45 mm; after that of *Acinobacter sp*, 40mm ; finally the least effect by *Pseudomonas aeruginosa* was 38mm).the DIZ values against (*E.coli*, *klebsiella pneumoniae*, *Acinobacter sp* , *Pseudomonas aeruginosa*) at concentration 50% was (45,40,30,30)mm Respectively .

The last concentration 25% and the DIZ values against (*E.coli*, *klebsiella pneumoniae*, *Acinobacter sp* , *Pseudomonas aeruginosa*) was (36 ,30,30,24)mm Respectively( table -3 &figure -2).

**Table3 Antibacterial activity of extract diluted with honey**

Organisms	Aqueous tomato extract diluted by honey			
	Diameter of inhibition zone in mm			
	25%	50%	75%	100% Honey
<i>Klebsiella sp</i>	30	40	45	55
<i>Escherichia coli</i>	36	45	50	60
<i>Acinetobacter</i>	30	30	40	49
<i>Pseudomonas sp</i>	24	30	38	50

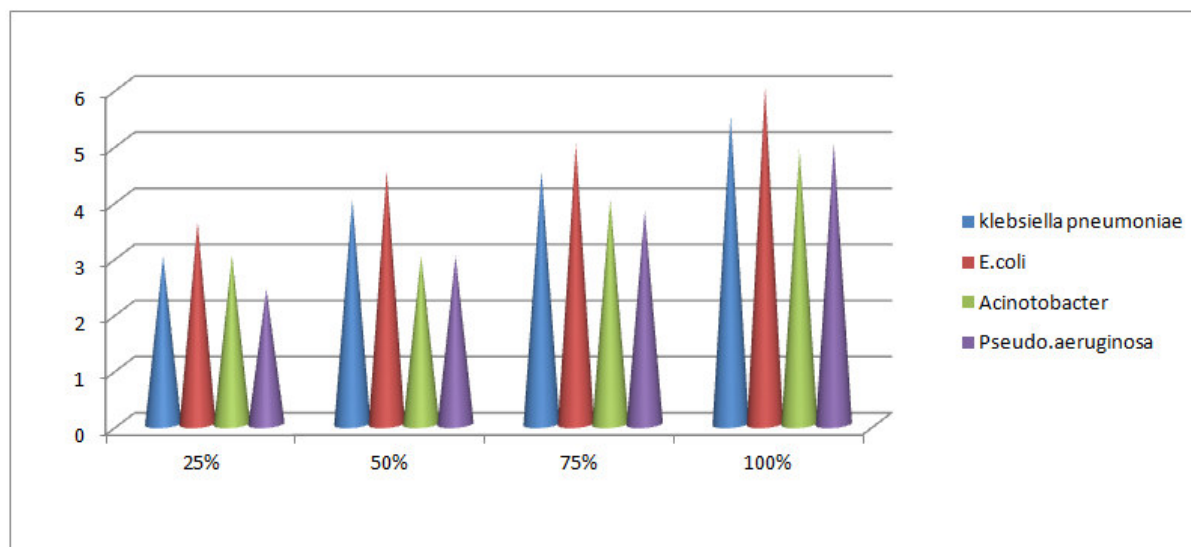


Figure 2

**Diameter of inhibition zones of aqueous tomato extract diluted with honey on selected bacterial isolates.**

**TABLE 4** Bacterial sensitivity to antibiotics

Bacteria isolates	Amoxicillin	Nitrofurantion	Levofloxacin	Ampicillin
<i>E.coli</i>	9	R	R	7
<i>Klebsiella pneumonia</i>	6	15	R	9
<i>Acinetobacter sp</i>	R	6	13	R
<i>Pseudomonas aeruginosa</i>	R	R	R	R

The employed commercial antibiotics ,antibacterial activities in some cases showed higher inhibitory potency and in other cases showed lower inhibitory potency than the extract., AMX, F,LF and AMP were the most potent on the test organisms. *Klebsiella* was the most inhibited (6-15 mm) by the reference drugs , the *Acinetobacter* was the followed by (6-13mm),the *E.coli* was (7-9mm) and the *pseudomonas* alone was resistant to all the employed reference drugs.

However, *E.coli* was resistant to F & LF ,*Klebsiella* was only resistant to LF and *Acinetobacter* showed resistant to AMX & AMP.

AMX, F, LF and AMP. AMX showed the least therapeutic effect (6-9 mm) on the test bacterial isolates, while the F appeared most effect (6-15mm) on the test bacterial isolates (Table 4).

**Discussion**

The antibacterial activity of extract (source) will differ , when source or the extract dilutes with other supported material ; It is possible Increases the effectiveness of antibacterial than soluble in a single solvent.

Tomatoes proved to be effective against microorganisms such as staphylococcus aureus , proteus ,bacillus& antifungal candida albicans, aspergillus niger(Sung *et al.*2007;Pavlovic *et al.*2013)

In this present study, tomato extract produces antimicrobial effect on some Gram-negative microorganisms selected , the results obtained showed antibacterial action is linked with the presence active compound of tomato extract on some of the isolated microbes *Escherichia coli* , *pseudomonas aeruginosa* ,*klebsiella pneumoniae* and *Acinetobacter sp* . but tomato extract appear high efficiency antibacterial properties when combined with other substance like the honey , the zone of inhibition was increase when the extract Diluted with honey.

Is due susceptibility tomato in the inhibition of different types of bacteria and fungi to contains these compounds acts as anticancer, anti- flammatory and antimicrobial activity(Unnisa *et al.*2012;Krishna *et al.*2013;Omodamiro *et al.*2013).

Effectiveness factor of the tomato is Lycopene that possesses antibacterial and antifungal properties(Dahan *et al.*2008;Rao,AV.2002).

Furthermore, the antibacterial activity of honey is attributed to the presence of “inhibin”, which acts as an antibacterial factor other than hydrogen peroxide. While in other products, several other factors play important roles like osmotic properties of honey which is saturated or super saturated solution of sugars with 84% being a mixture of fructose and glucose .There by, the inhibitory activity caused by the osmotic effect of honey dilutions obviously depends on the species of bacteria. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects(Moussa *et al.*2012)

the tomato extract when diluted with D.W showed antibacterial activity only at concentration 100% ,while when extract diluted with honey give us antibacterial activity at all concentrations that Mentioned

All bacterial isolates resistant (*Acinetobacter* ,*E.coli*, *Klebsiella pneumoniae* &*pseudomonas aeruginosa*) of diluted concentrations but showed sensitive at pure extract activity & when extract combine with honey.

In addition ,not all bacterial isolates showed sensitive to antibiotics (like *Klebsiella*) are explained by many option like: the nature of bacteria itself, physiological structure ,genetic mutation unlike effect the plant extract activity that expected to has target sites other than those used by antibiotic (Anjana *et al.*2009).

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