

## The Biological Activity of Tomato leaves Extract (*Solanum Lycopersicum*) against *Escherichia coli* Bacteria in vitro

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### ABSTRACT :

Tomato is the public surname for *Solanum lycopersicum* that belongs to family of *Solanaceae*. The aim of the present study to estimate the biological effect of tomato leaves extract on *Escherichia coli* and to identify on the best solvent gives the highest amount of extract. Tomato leaves (*Solanum Lycopersicum*) were collected of a farm near the Euphrates river in Ramadi city/Anbar governorate/Iraq. A 50 g of dried powder of tomato leaves were mixed (ratio 1/10) in glass beaker with 500 ml of solvent ( aqueous ,ethanolic , organic ). The extracts were filtered and placed into Petri dishes and incubate in oven under 50°C for three days to evaporate the solvents and get the extract (crude) and the zone of inhibition's diameter was measured to the closest millimeter (mm) during the screening of the anti-bacterial activity using the well diffusion method. The findings of these study demonstrate that aqueous solvent gave the highest amount of extract, recovery reached (21.84%), followed by the alcoholic extract (17.918%), while the lowest recovery amount of extract was in the organic solvent reached (3.642%). The present study revealed that tomato ethanolic extract gave the highest inhibition (22 mm) against *E.Coli* bacteria at 100 mg/dl concentration ,while the lowest inhibition recorded (9 mm) at 10 mg/dl concentration. Our study suggest that tomato leaves are consider important part of plant and it a potential source of natural antimicrobial factors.

**Keywords:** Tomato, *Solanum lycopersicum* , *Escherichia coli* , Zone inhibition ,Extract.

### الفعالية البيولوجية

### لمستخلص أوراق الطماطم على البكتيريا القولونية في المختبر

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### مستخلص

الطماطم هو الاسم العلمي لـ *Solanum lycopersicum* الذي ينتمي إلى عائلة *Solanaceae*. الهدف من الدراسة الحالية هو تقدير الفعالية البيولوجية لمستخلص أوراق الطماطم على البكتيريا القولونية والتعرف على أفضل مذيب من المذيبات الثلاث المستخدمة ( المائي ، الكحولي ، العضوي ) من حيث كمية الاستخلاص والحصول على أعلى كمية من المادة الخام من أوراق الطماطم. جمعت أوراق الطماطم من مزرعة محاذية لنهر الفرات في مدينة الرمادي / محافظة الانبار / العراق وتم تصنيفها في مركز دراسة الصحراء في جامعة الانبار للتعرف على هوية النبات بعد ذلك تم غسلها من التربة وتقطيعها وتجفيفها تحت أشعة الشمس غير المباشرة وتم سحقها للحصول على مسحوق ناعم وتم مزج 50 غم من المسحوق المجفف في بيكر زجاجي مع 500 مل من المذيب (مائي ، إيثانولي ، عضوي) بنسبة (1:10) بطريقة النقع. تم ترشيح المستخلصات ووضعها في أطباق بتري واحتضانها في الفرن تحت درجة حرارة 50 درجة مئوية لمدة ثلاثة أيام لتبخير المذيبات والحصول على المستخلص (الخام) ، واستخدمت طريقة الحفر بالأكار لقياس الفعالية البيولوجية للمستخلص الإيثانولي وتم قياس قطر التثبيط بالمليمتر . أظهرت نتائج هذه الدراسة أن المذيب المائي أعطى أعلى كمية من المستخلص ، حيث بلغت نسبة الاستخلاص (21.84٪) ، يليه المستخلص الكحولي (17.918٪) ، بينما كانت أقل كمية من المادة الخام في المذيب العضوي (3.642٪). كما أشارت الدراسة أن مستخلص الطماطم الإيثانولي أعطى أعلى تثبيط (22 ملم) ضد البكتيريا القولونية عند التركيز 100 ملغم / ديسيلتر ، بينما سجل أقل تثبيط (9 ملم) عند التركيز 10 ملغم / ديسيلتر. وتقتراح دراستنا إلى أن أوراق الطماطم تعتبر جزءاً مهماً من النبات وأنها مصدر محتمل للعوامل الطبيعية المضادة للميكروبات:

الكلمات المفتاحية : أوراق الطماطم ، المستخلص ، البكتيريا القولونية ، التثبيط البكتيري ، المذيبات .

## INTRODUCTION:

Tomato are the common name for (*Solanum Lycopersicum*) belongs to the Solanaceae family that comprises over 3,000 species, and considered essential vegetable economically, because to its (nutritious value, relish, accessibility, affordability), and rich in (vitamins, nutrients, phytochemicals and dietary fibers), for that noted an rise in demand in aggregate world production with rise 46.87 percent, which in 2001 about 108 million tons after that in 2008 the production exceeded 130 million tons then reached in 2011 to 159 million tons.[1] leaf curling, plant stunting and flower abscission. The current understanding of the host plant defense response to this virus is very limited. Using whole transcriptome sequencing, we analyzed the differential gene expression in response to TYLCV infection in the TYLCV-resistant tomato breeding line CLN2777A (R[2][3][4]. Tomato are a precursor source of antioxidants commonly used in Mediterranean diet, along with carotenoids, other antioxidant compounds present in tomato like (tocopherols, phenols and ascorbic acid) play a crucial function to prevention diseases[5]the antioxidant compo-

nents and of six high-lycopene (Lyco 1, Lyco 2, HLY 02, HLY 13, HLY 18 and Kalvert. These species of plants are source of bioactive chemicals and rich in (terpenoids, phenolic compounds, and other secondary metabolites) with biological activities (anti-proliferative, anti-mutagenic, chemopreventive, anti-inflammatory properties and antigenotoxic) and each on have advantages of human health [6].

Tomatoes produce great amount of C-aminobutyric acid at fruit development, despite presence rich nutrients (such as alkaloids, Vitamin E, Vitamin C, and lycopene)[7]. In the body the tomato has detoxification influence, may be because existence of chlorine and sulfur in tomato sulfur in tomato keeps the liver from cirrhosis [8]. Regardless of their nutritional value, tomatoes (*Solanum lycopersicum*) was confirmed to have cardioprotective impact at the endothelium and platelet levels, whether they are fresh or processed (such as tomato paste)[9]. Studies showed a significant converse connections between tomato consuming and danger of particular kinds of cancer, age-related macular degeneration and cardiovascular diseases, due to its contain the mixture balanced of min-

eral compounds and antioxidants such as (alcohol ,fatty acid, tocopherol and phenolic compounds, vitamins E and C, lycopene,  $\beta$ -carotene , lutein, flavonoids like quercetin, and hydroxycinnamic acid derivatives)[10][11][12][13].

The one of major issues of the tomato industry in the wide world are diseases , and the sensitivity of tomato to numerous pathogenic microorganisms causes to a strong utilize of agrochemicals[14]. The chlorophyll content in plant denotes to plant veracity and is highly linked to its physical status[15]. Tomato supply a unique species of phytonutrients .Its high extremely source of enzyme-promoting molybdenum, heart-healthy potassium, blood sugar-balancing manganese, heart-healthy magnesium, vitamin B6, folate, ,dietary fiber, niacin, phosphorus, vitamin E, vitamin B1, energy-producing iron, bone healthy copper and muscle building protein [16]. Microorganisms are existence in nature in everywhere .Gram negative pathogens chiefly *Escherichia coli* is found in the environment in anywhere almost and remains discriminately one of the main reasons for contamination of food [17], *Klebsiella pneumoniae* and *Escherich-*

*ia coli* are reported as causal factors of foodborne diseases, which existing in various sources especially raw vegetables[18].

## MATERIALS AND METHODS

### Source and identification of plant (Scientific classification)

Tomato leaves plant (*Solanum Lycopersicum*) collected of a farm near the Euphrates river in AL-Ramadi-Anbar governorate/Iraq .The plant was identified at the Center of Desert Studies Herbarium- University of Anbar as the following :

Division : Spermaphyta

Subdivision : Angiospermae

Class : Dicotyledones

Order : Solanales

Family :Solanaceae

Genus : Lycopersicon

Species :Lycopersicon , Esculentum

### Preparation of Extract

The leaves of the tomato plant were cutting and washed to rid it of dust and then dried under indirect sunlight and crushed into powder by using the pestle and mortar and put in dry and clean bags until use. A 50 g of dried powder tomato leaves were weighed and mixed with 500 ml of solvent (ratio 1/10) in

glass beaker with closed the top with aluminum foil to prevent the evaporation and then shaken by using magnetic stirrer, the extraction was performed three times for three solvents ( aqueous, ethanolic, organic ). The extracts were filtered through Whatman (No.1) filter paper under rarefied pressure, the solution containing extract were placed into petri dishes and incubating in oven under 50°C for three days to evaporated the solvents and concentrated the extract (crude). The dried extracts were collected in plastic container and weigh before and after putting the extracts to determine the percentage for each extract and kept in airtight containers at 4°C until performed the antimicrobial activity.

### Antimicrobial assays.

A female patient with an acute and recurring urinary tract infection was the source of the pathogenic bacteria at Al-Ramadi Teaching Hospital in Iraq. The technique relies on dilution and was created by employing ethylene glycol, a solvent that is inert to microorganisms[19], and using ethylene glycol to reduce the quantity to 2 ml to get the final concentrations of (1–10%), repeated concentrations of the extract

from (10–100 mg) were used.

Using the well diffusion technique, antibacterial activity was tested [20]. The standardized bacterial inoculums were seeded onto the Mueller-Hinton agar plates in a volume of (0.1ml). With a clean glass spreader, the inoculums were equally distributed across the plate. For 20 minutes, the seeded plates were allowed to dry at 37 °C in the incubator. On the surface of the plates, standard wells were cut with a (9 mm) crack edge, and (0.1 ml) of each concentration was added as a control along with ethylene glycol. The infected discs were incubated for 24 hours at 37°C, and the width of the zone that was inhibited was measured to the closest millimeter (mm).

### RESULTS AND DISCUSSION:

The tomatoes leaves and stems are important sources used as antimicrobial agents due to contain the functional constituents [21]. The findings of recent study demonstrated that aqueous solvent is the highest solvent in extracting tomato leaves as its recovery reached (21.84%), followed by the alcoholic extract where the recovery concentration was (17.918%), while the lowest amount of extract was in the

organic solvent reached (3.642%) as shown in table (1). The highest amount recovery by aqueous solvent may be due to its low volatility and is not affected much by low temperature that used for extraction and does not interfere with other materials and affect their efficiency. While organic solvent (chloroform) is fast volatilization which reduces the efficiency of process

plant extraction. According to a few studies, choosing the right solvent is crucial since antimicrobial compounds have varying solubilities relying on the solvent polarity[22]. Previous study mentioned that the ability extraction of phytochemical compounds is vary from solvent to solvent according to their polarities [23].

**Table (1): The percentages of *Solanum Lucopercicum* recovery by different solvents**

Type of Solvent	Wt. of extract in( g )	Percentage of extract %
Aqueous solvent (Water)	<b>10.920 g</b>	<b>21.84 %</b>
Alcoholic solvent (85% (ethanol)	<b>8.959 g</b>	<b>17.918 %</b>
Organic Solvent (Chloroform)	<b>1.821 g</b>	<b>3.642 %</b>

The present study revealed that ethanolic tomato leaves extract gave the highest inhibition (22 mm) against *E.Coli* bacteria at 100 mg/dl concentration ,while the lowest inhibition recorded (9 mm) at 10 mg/dl concentration as shown in table (2) and figure (1). These findings agrees with previous study done by [16] and [24] which reported the highest inhibition zone at high concentration 100 mg/ml and highest inhibition zone showed in gram negative bacteria as compared with gram positive bacteria. Previous study

reported that the extracts are more active when concentrations are high and inactive when the concentrations very low so that the present study proposes that the bacterial growth effectiveness inhibition of the extracts is dose dependent [23]. These study are accordance with another study done on the deferent parts of two tomato varieties ,The maximum antibacterial activity was demonstrated by the leaf extracts in both types, followed by the stem and whole-plant extracts. Increased polyphenol levels were favourably linked



with *E. coli*-specific antibacterial activity[25]. According to earlier studies, the tomato's non-edible portions contain more antibacterial compounds than its edible portions. For instance, compared to tomato fruits, tomato leaves have elevated concentration of antimicrobial metabolites such as (caffeic acid, chlorogenic acid, vanillic acid, sabinene, terpinene, phellandrene, tomatine, and dehydro-tomatine)[22]. The antibacterial efficiency of the extracts may be return to found constituents such as (flavonoids, carbohydrates, alkaloids, saponins, tannins, glycosides, phytosterols, steroids, triterpenoids, and cardiac glycosides) identified in aqueous and ethanolic tomato leaves extracts[26].

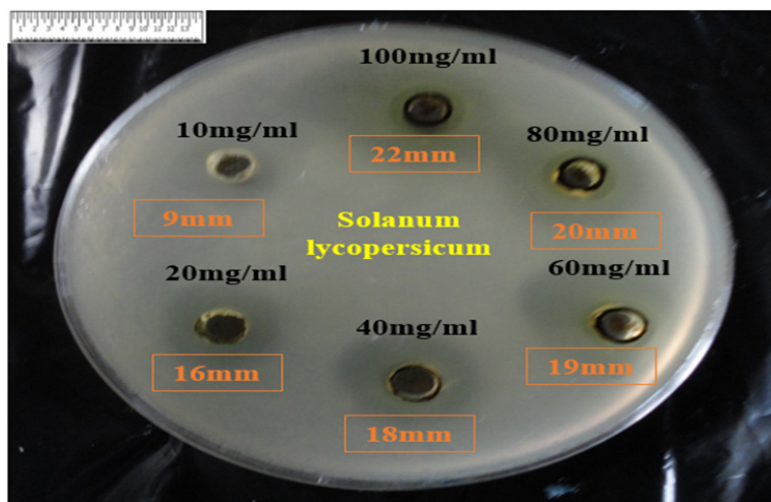
with *E. coli*-specific antibacterial activity[25]. According to earlier studies, the tomato's non-edible portions contain more antibacterial compounds than its edible portions. For instance, compared to tomato fruits, tomato leaves have elevated concentration of antimicrobial metabolites such as (caffeic acid, chlorogenic acid, vanillic acid, sabinene, terpinene, phellandrene, tomatine, and dehydro-tomatine)[22]. The antibacterial efficiency of the extracts may be return to found constituents such as (flavonoids, carbohydrates, alkaloids, saponins, tannins, glycosides, phytosterols, steroids, triterpenoids, and cardiac glycosides) identified in aqueous and ethanolic tomato leaves extracts[26].

**Table (2) The zone inhibition of *Solanum Lycopersicum* against *E. Coli* bacteria**

Con.(mg/ml) Zone of inhibition (mm)	10mg/ ml	20mg/ml	40mg/ml	60mg/ml	80mg/ ml	100mg/ml
<i>E.coli</i>	9	16	18	19	20	22
DMSO (Control)	0	0	0	0	0	0

The results of another study investigated against *Escherichia coli* by dilution method showed that the minimum inhibition concentration (MIC) of the tomato extract was comparable to the antibiotic ciprofloxacin[27]. While another study revealed that the extract of tomato showed high efficacy antibacterial characteristics when incorporated with another materiality such as honey, so that when the extract diluted with honey the inhibition zone was raised[28]. The activity agent of tomato leaves is because the existence of lycopene material which have antibacterial

and antifungal characteristics[23]. Previous study reported that extraction of lycopene from tomato pomace in traditional and nano-form as nano-particles, show antioxidant activity. Furthermore, it has an antimicrobial activity on every tested bacteria. As well as, the anticancer effectiveness against several cell line like MCF-7, HepG-2, and HCT-116 was observed[29]. Lycopene is prosperous in tomato and a non-enzymatic antioxidant. It's considered a powerful antioxidant and much helpful in ejecting singlet oxygen, causing in oxidative stress [30].



**Figure (1): The inhibition zone of *Solanum Lycopersicum* in different concentrations against *E. Coli***

The results of study done by Natividad L and Rafael R , (2014) are not consistent with our findings which are indicate the tomato extract can't inhibit the growth of *E. coli*. This variance can be regarding to the indignity of the tomato strain, the kind of extract, and the kind of standard bacterial strain which are examined in current study[27][31].

## CONCLUSION

Tomato leaves (*Solanum Lycopersicum*) are effectiveness factor because the existence of lycopene that have antifungal and antibacterial characteristics. This study demonstrated that aqueous solvent gave the highest amount of extract (crude material) followed by the alcoholic extract while

the lowest amount of extract was in the organic solvent. The ethanolic of tomato leaves extract was used for antimicrobial activity against *Escherichia Coli* bacteria where noted the highest inhibition in concentration 100 mg/dl which gave the zone inhibition (22 mm). Accordingly, the study contends that the extract dosage determines how much bacterial growth is inhibited. According to this study, tomato leaves are a significant component of the plant and a possible source of naturally occurring antibacterial substances. This activity is explained by the leaves of plant richness in phenols, flavonoids, alkaloids, and tannins etc.

**Conflicts of interest:** Nil

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