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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 اعلى كمية من المادة الخام من اوراق الطماطم. جمعت اوراق 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 الاتربة وتقطيعها وتجفيفها تحت اشعة 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 البايلوجية للمستخلص الايثانولي 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 اشارت الدراسة أن مستخلص الطماطم الإيثانولي أعطى أعلى تثبيط (22 ملم) ضد البكتريا القولونية عند التركيز 100 ملغم / ديسيلتر ، بينما سجل أقل تثبيط (9ملم) عند التركيز 10 ملغم/ ديسيلتر. وتقترح دراستنا إلى أن أوراق الطماطم تعتبر جزءًا مهمًا من النبات وأنها مصدر محتمل للعوامل الطبيعية المضادة للميكروبات:

الكلمات المفتاحية : أوراق الطماطم ، المستخلص ، البكتريا 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][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 components 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 un 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 mineral compounds and antioxidants such as (alcohol ,fatty acid, tocopherol and phenolic compounds, vitamins E and C, lycopene, β-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]among which Proteobacteria was the most represented (90%. 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 Escherichia 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
Gunus : 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 magnet-

ic 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)	**10.920 g**	**21.84 %**
Alcoholic solvent (85% (ethanol)	**8.959 g**	**17.918 %**
Organic Solvent (Chloroform)	**1.821 g**	**3.642 %**

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].

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

Con.(mg/ml) / Zone of inhibition (mm)	10mg/ml	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml
E.coli	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].

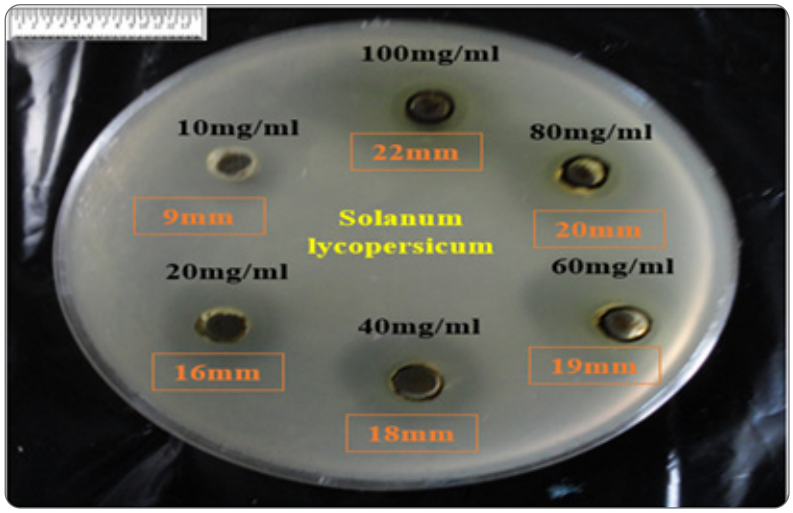

Figure (1): The inhibition zone of Solanum Lucopercicum in different concentrations against E. Coli

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 Lucopercicum) 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 concentration100 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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REFERENCES:

[1] T. Chen et al., "Comparative transcriptome profiling of a resistant vs. susceptible tomato (Solanum lycopersicum) cultivar in response to infection by tomato yellow leaf curl virus," PLoS One, vol. 8, no. 11, pp. 4–6, 2013.

[2] S. M. Devadas, S. R. Giffen, N. Kumar, R. Lobo, and M. Ballal, "Activity of Solanum lycopersicum against Candida species isolated from retro-positive patients – An invitro study," J. Pharm. Sci. Res., vol. 9, no. 7, pp. 1233–1236, 2017.

[3] M. Taveira et al., "Lycopersicon esculentum Seeds: An industrial byproduct as an antimicrobial agent," J. Agric. Food Chem., vol. 58, no. 17, pp. 9529–9536, 2010.

[4] F. A. Obeng, P. B. Gyasi, M. Olu-Taiwo, and F. P. Ayeh-Kumi, "Microbial Assessment of Tomatoes (Lycopersicon esculentum) Sold at Some Central Markets in Ghana," Biomed Res. Int., vol. 2018, 2018.

[5] R. Ilahy, C. Hdider, M. S. Lenucci, I. Tlili, and G. Dalessandro, "Phytochemical composition and antioxidant activity of high-lycopene tomato (Solanum lycopersicum L.) cultivars grown in Southern Italy," Sci. Hortic. (Amsterdam)., vol. 127, no. 3, pp. 255–261, 2011.

[6] S. Kainat et al., "Determination and Comparison of Phytochemicals, Phenolics, and Flavonoids in Solanum lycopersicum Using FTIR Spectroscopy," Food Anal. Methods, vol. 15, no. 11, pp. 2931–2939, 2022.

[7] R. Li et al., "Multiplexed CRISPR/Cas9-mediated metabolic engineering of γ-aminobutyric acid

levels in Solanum lycopersicum," Plant Biotechnol. J., vol. 16, no. 2, pp. 415–427, 2018.

[8] P. Shukla, K. Bajpai, S. Tripathi, and G. K. Gautam, "A Review on the Taxonomy , Ethnobotany , Chemistry and Pharmacology of Solanum Lycopersicum Linn," Int. J. Chem. Pharm. Sci., vol. 1, no. 8, pp. 521–527, 2013.

[9] E. Fuentes et al., "Bioassay-guided isolation and HPLC determination of bioactive compound that relate to the antiplatelet activity (adhesion, secretion, and aggregation) from solanum lycopersicum," Evidence-based Complement. Altern. Med., vol. 2012, 2012.

[10] M. Dorais, D. L. Ehret, and A. P. Papadopoulos, "Tomato (Solanum lycopersicum) health components: From the seed to the consumer," Phytochem. Rev., vol. 7, no. 2, pp. 231–250, 2008.

[11] Y. Ma et al., "Components, antioxidant and antibacterial activity of tomato seed oil," Food Sci. Technol. Res., vol. 20, no. 1, pp. 1–6, 2014.

[12] K. Szabo, Z. Diaconeasa, A. F. Cătoi, and D. C. Vodnar, "Screening of ten tomato varieties processing waste for bioactive components and their related antioxidant and antimicrobial activities," Antioxidants, vol. 8, no. 8, 2019.

[13] A. Gerszberg, K. Hnatuszko-Konka, T. Kowalczyk, and A. K. Kononowicz, "Tomato (Solanum lycopersicum L.) in the service of biotechnology," Plant Cell. Tissue Organ Cult., vol. 120, no. 3, pp. 881–902, 2015.

[14] F. M. Romero, M. Marina, and F. L. Pieckenstain, "The communities of tomato (Solanum lycopersicum L.) leaf endophytic bacteria, analyzed by 16S-ribosomal RNA gene pyrosequencing," FEMS Microbiol. Lett., vol. 351, no. 2, pp. 187–194, 2014.

[15] K. Durairaj et al., "Characterization and assessment of two biocontrol bacteria against Pseudomonas syringae wilt in Solanum lycopersicum and its genetic responses," Microbiol. Res., vol. 206, no. July 2017, pp. 43–49, 2018.

[16] O. D. Omodamiro and U. Amechi, "The phytochemical content, antioxidant, antimicrobial and anti-inflammatory activities of Lycoper-

sicon esculentum (Tomato)," Dep. Biochem. Coll. Nat. Sci. Micheal Okpara Univ. Agric. Umudike, Abia state Niger., vol. 3, no. 5, pp. 70–81, 2013.

[17]J. M. Oduwaiye, I. A. Dan-ologe, and J. A. Obadipe, "Original Article Solanum Lycopersicum Phenotypes Juice Potentials : Its Antimicrobial Investigation Food Preservative," pp. 239–251, 2022.

[18]C. Y. Muñoz, L. Zhou, Y. Yi, and O. P. Kuipers, "Biocontrol properties from phyllospheric bacteria isolated from Solanum lycopersicum and Lactuca sativa and genome mining of antimicrobial gene clusters," BMC Genomics, vol. 23, no. 1, pp. 1–12, 2022.

[19]F. D. Charles, O. W.; Ole, G. & Robert, Textbook of organic medicinal and pharmaceutical chemistry. 1969.

[20]P. Saeed, S. & Tariq, "Anti-bacterial activities of Menthapiperita, Pisumsativum and Momordicacharantia," Pak. J. Bot., vol. 37, pp. 997–213., 2005.

[21]F. Kobayashi, K. Ishida, H. Ikeura, S. Odake, and Y. Hayata, "Application of Tomato (Solanum lycopersicum) Leaf Volatiles as Antifungal Agents against Plant Pathogenic Fungi," J. Agric. Sci., vol. 4, no. 8, pp. 231–235, 2012.

[22]D. S. Kim, Y. Kwack, J. H. Lee, and C. Chun, "Antimicrobial activity of various parts of tomato plants varied with different solvent extracts," Plant Pathol. J., vol. 35, no. 2, pp. 149–155, 2019, doi: 10.5423/PPJ.OA.07.2018.0132.

[23]M. Umar et al., "Evaluation of Phytochemical and in vitro Antimicrobial Effects of Solanum lycopersicum Linn. (Tomato) on Oral Thrush and Human Cariogenic Pathogens," J. Adv. Med. Pharm. Sci., vol. 11, no. 4, pp. 1–9, 2016.

[24]B. K. and J. D. Murugesana M, "Phytochemical , antioxidant and antimicrobial analysis of solanum lycoperersicum esculentum leaf extract," Int. J. Pharm. Biol. Sci., vol. 8, no. 4, pp. 1147–1152, 2018.

[25]N. P. Silva-Beltrán et al., "Total-Phenolic,Flavonoid,Tomatine,and Tomatidine Contents and Antioxidant and Antimicrobial Activities of Extracts of Tomato Plant," Int. J. Anal. Chem., vol. 2015, pp. 1–10, 2015.

[26]A. Maz'uma, "Antibacterial Activity of <i>Citrus sinensis</i> and <i>Solanum lycopersicum</i> on Wound Isolated from Hospitals in Kaduna Metropolis Kaduna Nigeria," Int. J. Biomed. Mater. Res., vol. 6, no. 2, p. 40, 2018.

[27]M. Shamshirgaran et al., "Antibacterial effects of the aqueous extract of Lycopersicon esculentum mill native in South Khorasan of Iran against four species associated with gastrointestinal infections," J Bas Res Med Sci, vol. 7, no. 3, pp. 1–6, 2020.

[28]R. M. Al-oqaili, B. Basim, M. Muhammed, and A. Salman, "In Vitro Antibacterial Activity of Solanum Lycopersicum Extract against some Pathogenic Bacteria," Food Sci. Qual. Manag., vol. 27, pp. 12–18, 2014.

[29]A. A. Khesbak, "Studies on Biochemical Changes and Prolactin Level Evaluation in Patients with Chronic Kidney Disease," 2019.

[30]A. H. Henderson, I. N. E. Lister, E. Fachrial, and E. Girsang, "Antioxidant and Anti-Elastase Activity of Ethanol Extract of Tomato (Solanum lycopersicum L.)," Bul. Penelit. Tanam. Rempah dan Obat, vol. 31, no. 2, p. 67, 2020, doi: 10.21082/bullittro.v31n2.2020.67-74.

[31]Natividad LR and Rafael RR, "Carotenoid Analyses and Antibacterial Assay of Annato (Bixa orellana L.), Carrot (Daucus carota L.), Corn (Zea mays L.) and Tomato (Solanum lycopersicum L.) Extracts," Res. J. Recent Sci., vol. 3, no. 3, pp. 40–45, 2014.