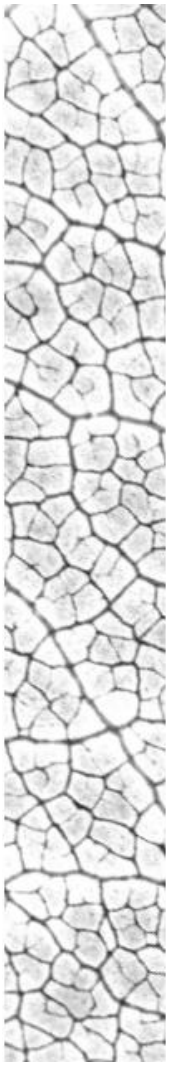

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Investigation the antimicrobial and antioxidant activity of lycopene extraction from *Solanum Lycopersicum*

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Abstract
The red color in tomatoes because of the presence of lycopene carotenoid. a simple, effective technique for solvent extraction the lycopene from tomato also varied range of 50 different solvents systems have been used to be the best solvent for extraction, subsequent in the separation of extra lycopene than other solvents. An early method with several alteration for the lycopene extraction and description was used. Identification as well as examine the antimicrobial activity using four concentration (25,50,75 and 100 mg/ml), more action was revealed in the highest concentration (100 mg) in all types of bacteria (*Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa, Escherichia coli and Candida albicans*) that used, but *Pseudomonas aeruginosa* was the utmost affected than the rest. Finally, in antioxidant assay the DPPH radical scavenging activity used in two concentrations of lycopene extract (0.4 and 0.8 mg/ml) compared to the ascorbic acid as a standard. The results showed lycopene had great antioxidant effectiveness.

Keywords: Lycopene, antimicrobial activity, antioxidant activity, DPPH radical scavenger

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INTRODUCTION

Tomatoes have a varied range of antioxidants containing Vitamin E & C, carotenoids, flavonoids also phenolic compounds. It is furthermore one of the most copious non-vitamin equivalents existing in human blood from food intake (Sathish et al. 2009). This defensive effect has been documented to carotenoids, which are exclusive of the chief classes of phytochemicals in this plant (Khachik et al. 2002). A carotenoid pigment, embraces the ability to controller numerous diseases such as cancer also other progressive syndromes by delaying the action of free radicals (Nasir 2014). Among of the quantity of carotenoids, lycopene is unique and only of the extreme impact initiate in tomato, watermelon as well as rosy grape fruit giving them a specific red coloration. Newly, lycopene has been established as in general known as safe in US (Crandall, 2003). The best essential sources of lycopene are tomatoes and tomato produces, this pigment can characterize about 85% of exclusively as a carotenoids existing in the fruits; its concentration can diverge from 30 to 200 ppm in the fresh crops or from (430- 3000) ppm on dry source (Leoni 1993). A sum of extraction as well purification procedures can be beneficial to achieve this product for instance solvent extraction (Zelkha et al. 1989). The optimal solvents; ethyl acetate also diethyl ether are non-toxic, appropriate as additives in the U.S. of FDA, biodegradable and have moderately high flash point (Strati and Oreopoulou 2011). The main target of this work is to suggest wide range of solvents systems and an old method with some modification for the lycopene extraction from tomato then characterization, identification and investigate the antimicrobial also antioxidant activity.

MATERIALS AND METHODS

Tomatoes are purchasing from locally market at Basrah, Iraq. Tomatoes collected during summer 2016. It was identified as Lycopersicum esculentum Mill (Solanaceae), they were washed with water to remove the soil also dust particles, the surface sterilized using 70% alcohol then cleaned with sterile water. They were dried in air oven (Caplain Oven FRP 4/8, France) after that fruits were cut in the form of cubes measured (10*10*10mm3) at temperature 80° for two h then reducing the heat to 60° for 6 h (Ching -Hui et al. 2006). it was ground automatically according to Abdul-Jawad et

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al (2009), later packed in opaque packing bags and kept at room temperature. All solvents (analytical and uvasol) used to extract lycopene: hexane, ethanol, methanol, chloroform, acetone, carbon tetrachloride, benzene, diethyl ether, ethyl acetate while dim-ethylformamide and acetonitrile of HPLC grade; all these diluents were achieved by Merck (Darmstadt, Germany). All plants and bacteria were used for experiments and were approved by the Research Ethical Committee of Basrah University.

Lycopene quantity measurement

The appropriate solvent aimed to effective lycopene extraction from tomatoes, in cooperation the polarity of the solvents were a selection of, such as hexane,acetone, diethyl ether, methanol, ethanol, ethyl acetate, Carbone tetrachloride, chloroform and benzene. unisolvent, multi solvents system (1:1) in addition to tripartite solvents system (1:1:1) existed. The superlative solvents system were recognized on utmost percentage of lycopene retrieval in the tomatoes sample. Lycopene was used spectrophotometer method to measurement at 503 nm(Barba et al. 2013). The data were recorded and the lycopene content (mg/100g) was calculated using the following formula of Lavecchia and Zuorro (2008)

A503 V 100

Lycopene content**----*----------* ---------- = -----**

a 503 1000 W

A503 represented of absorbance at 503nm

a503 represented précis elimination coefficient of lycopene in n-hexane

v represented total volume of solution per ml**.**

w represented weight per gram in tomato sample**.**

Simple Solvent Extraction

Lycopene was extracted according to Shi method (Shi et al. 1999), where has been weighed 10 gm From dried tomatoes by electronic balance (AND,400,Korea) and put in kimax tube with screw cap, it was completely covered with aluminum foil to prevent exposure to light, then prepared 100 ml from mixture of solvents: acetone, hexane and ethanol (1:2:1) respectively, at that time were add to sample of tomatoes and shaken with an electric vibrator (Vortex-2Genie,G-5-60E,USA) for 30 min, it were left for 2 min with add ten ml of DW where two layer separated, the layer of hexane was collected in the tube of 50 ml, soap was removed by adding 1.5 g of KOH in 100 ml DW, separating the lycopene layer and filtering the solution under vacuum, it was put in clean Petri dish and dried using anhydrous calcium chloride by vacuum desiccators.

Identification of Lycopene

Lycopene in all samples was determined by TLC then HPLC. The TLC (Silica gel 60 F254) Aluminum plate was used for separating and purifying extract of lycopene (20×20 cm) were stimulated at 110 C0 for 30 min then used as a stationary phase to determine the lycopene in the resulting extract. Two solvents were used as the mobile phase on the TLC plate's toluene-hexane (1:19 v/v). Moreover, the amount of lycopene extracted from tomato was estimated using spectrophotometer (Chrom Tech,USA) at wavelength 503 nm using n-hexane as blank and the amount of lycopene was deliberate by lycopene destruction coefficient (E%) it's about 3150 depending on Chang and Liu (2007). Lycopene purity was estimated by HPLC by DAD-HPLC apparatus (LC 100UV 100 plus/Angstrom, USA,N11091250). The operating conditions of the device were as following: column was provided it Chromolith RP−C18E, 4.6 × 10 mm, injection volume (50 µl),at temperature (30 C0), the flow rate was (1 cm3 min−1), wave length (472 nm), the solvents that were used dimethylformamide A (60% DMF) and acetonitrile B (40%MeCN) used solvent B.

Anti-microbial Assay

The antimicrobial activity for lycopene extract was resolute using the well diffusion technique for antibacterial also antifungal activity (Schinor et al. 2007). Muller Hinton Agar was prepared, Using a sterilized cork borer for making the wells of 5mm in diameter on an agar plates after inoculating the agar plates with microorganisms. A 100 µl of lycopene extract from four concentration (25,50,75 and 100 mg/ml of Di methyl Sulfoxide (DMSO) was pipette into the wells. The plates were allowed on the counter for 30 min for pre diffusion of the extract to take place. The strains that were used for the assay *Staphylococcus aureus (S.aureus), Streptococcus pyogens (S. pyogens), Pseudomonas aeruginosa(P. aeruginosa), Escherichia coli (E. coli) and Candida albicans(C. albicans),* the treated Petri dishes were incubated overnight at 37C0for 24 h.

In Vitro Antioxidant Assay (DPPH)

An aptitude of lycopene extract to scavenge DPPH (Sigma- Aldrich Co, St. Louis, U.S.A.), the radicals were assessed according to Gyamfi et al. (1999). A drop of the radical was resolute through calculating the absorption at 517 nm. A radical scavenging action (RSA) was designed as a ratio of DPPH via the equation:

RSA% = [(a DPPH – a S)/ a DPPH]* 100

where (a S) is the absorbance of the solution next the sample extraction is added at a specific levels, also a DPPH is the absorbance of DPPH solutions. Moreover, ascorbic acid (S.D. Fine Chem., Biosar, India) was used as standards.

The statistical analysis

Entirely documents were exposed to investigation of difference (frequent measurement ANOVA) through using the SPSS (Version 9.05, Chicago, IL, U.S.A). Significantly variances among sample resources was evaluated by Duncan's multiple comparison test within $p < 0.05$.

Table 1. The lycopene Content Via Different Solvents Systems

No.	Solvent system	Lycopene content after 1h	Lycopene content after 2h	No.	Solvent system	Lycopene content after 1h	Lycopene content after 2h
1	hexane	0.120	0.469	26	diethyl ether: chloroform	0.420	0.654
2	ethanol	0.173	0.339	27	diethyl ether: benzene	0.243	0.291
3	methanol	0.173	0.182	28	diethyl ether: hexane	0.430	0.489
4	chloroform	0.193	0.889	29	diethyl ether: ccl_4	0.680	0.350
5	acetone	0.259	1.492	30	Diethyl ether: methanol	0.897	0.450
6	Carbone tetrachloride	0.263	0.876	31	Diethyl ether: chloroform	0.820	0.840
7	benzene	0.188	0.742	32	Diethyl ether: Benzene: hexane	0.315	0.410
8	methanol: ethanol	0.201	0.214	33	Diethyl ether: Benzene: chloroform	0.515	0.570
9	acetone: ethanol	0.585	0.460	34	ethyl acetate	1.090	0.375
10	methanol: acetone: ethanol	0.268	0.243	35	ethyl acetate: acetone	1.390	2.528
11	chloroform: methanol	3.000	3.000	36	ethyl acetate: ethanol	2.050	2.621
12	benzene: ethanol	2.915	3.000	37	ethyl acetate: methanol	0.205	0.281
13	benzene: chloroform	1.010	1.212	38	ethyl acetate: hexane	0.270	0.244
14	ccl_4:acetone: hexane	0.428	0.282	39	ethyl acetate: chloroform	1.150	0.583
15	hexane: ethanol	0.738	1.670	40	ethyl acetate: benzene	1.180	0.244
16	hexane: acetone: ethanol	2.824	0.331	41	ethyl acetate: diethyl ether	0.403	0.891
17	chloroform: hexane	0.736	1.059	42	ethyl acetate: acetone: hexane	0.380	0.259
18	ccl_4:ethanol	3.000	3.000	43	ethyl acetate: acetone: chloroform	1.180	0.580
19	ccl_4:acetone	0.850	0.735	44	ethyl acetate: diethyl ether: acetone	0.940	2.390
20	benzene: ccl_4	0.500	0.532	45	ethyl acetate: diethyl ether: ethanol	1.903	2.902
21	chloroform: ccl_4	0.430	0.482	46	ethyl acetate: diethyl ether: methanol	2.305	0.809
22	diethyl ether	0.289	0.529	47	Benzene: Acetone: Ethyl acetate	0.561	1.283
23	hexane: diethyl ether: acetone	0.202	0.271	48	ethyl acetate: chloroform: ethanol	2.180	2.541
24	diethyl ether: acetone	1.537	1.169	49	ethyl acetate: benzene: ethanol	0.497	0.851
25	diethyl ether: ethanol	0.790	0.785	50	ethyl acetate: hexane: ethanol	2.117	2.090

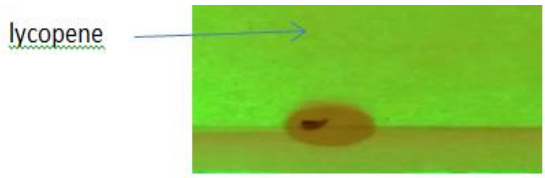

Fig. 1. TLC of lycopene, TLC plates was established with toluene: hexane (1:19, v/v)

RESULTS

Identifications of appropriate solvents system

The lycopene extract was examined for its contents with UV-Visible Spectroscopy. The investigates were completed for three times also typical value is defined in **Table 1**.

Identification of Lycopene in Simple Solvent Extraction

Isolate and purify lycopene extracted in simple solvent used thin layer chromatography silica gel plates. lycopene acyclic spot in **Fig. 1** appeared on the TLC plate with (RF = 0.4). The investigation of the lycopene extracted in simple solvent for the tomato by HPLC (isocratic analysis), is given in **Fig. 2**.

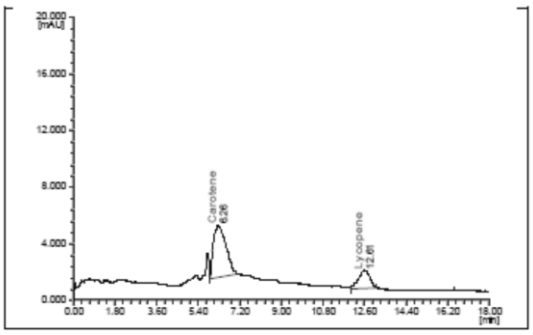

Fig. 2. HPLC Chromatogram of the lycopene extracted simple solvent for local tomato by Merck (Germany) Chromolith RP−C18E, 4.6 × 10 mm column, injection volume(50 µl), temp. (30 C^0), flow rate (1 cm^3 min^{-1}), wave length (472nm) solvent A (60% DMF) / solvent B (40%MeCN)

The single peak without any overlap and less than 1 min width at the retention time 12.61 min was considered to be the lycopene compound. The peak at the retention time 6.26 min was considered to be one of the β-carotenoid compounds. It is should be noted that the retention time of lycopene peak was indicative of the successful separation for the compound using DMF

Table 2. Antibacterial activity of lycopene extracted by simple

Organisms	Inhibition Zone(mm) In 25 mg/10ml DMSO	Inhibition Zone(mm) In 50mg/10ml DMSO	Inhibition Zone(mm) In 75mg/10ml DMSO	Inhibition Zone(mm) In 100mg/10ml DMSO
S. pyogens	5	5	6	7
E. coli	5	5	5	6
S. aureus	5	5	6	8
P. aeruginosa	6	7	9	12
C. albicans	5	6	7	9

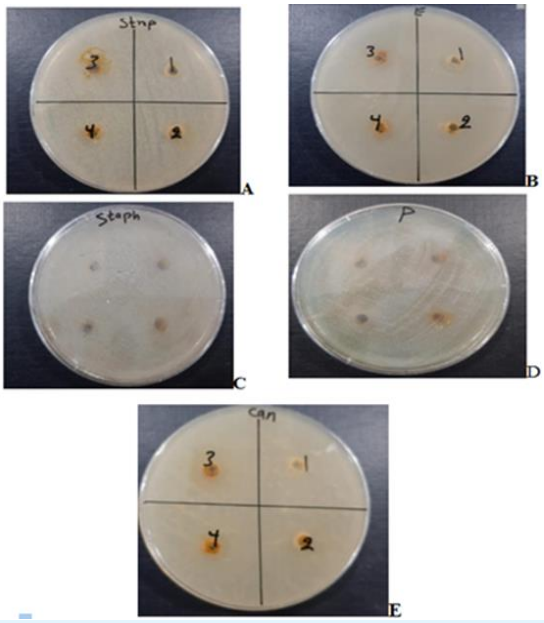

Fig. 3. Anti-microbial activity to lycopene extract against to A:*S. pyogens, B: E. coli C: S. aureus, D:P. aeruginosa, E:C. albicans.* scavenger activity of Lycopene extract

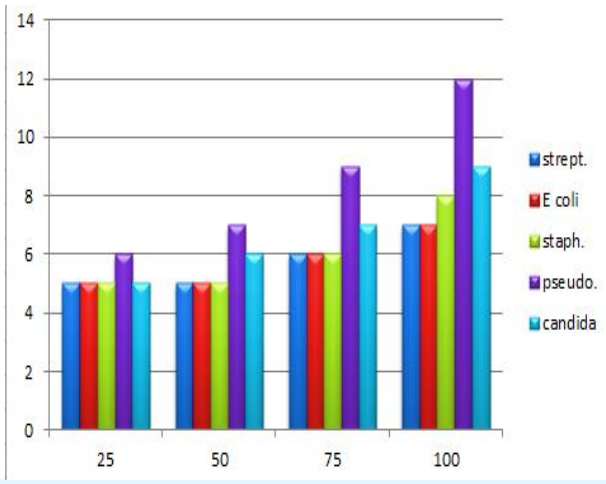

Fig. 4. Inhibition zones (mm) of lycopene extracted (concentration 25,50,75 and 100 mg) by simple solvent on certain bacteria

(dimethylformamide and MeCN (acetonitrile) as a stock solution and HPLC solvents (60% / 40%).

Antimicrobial activity

There was alteration in the antibacterial activity of lycopene extract from tomato by simple solvent. More activity was shown in the highest concentration (100 mg) in each type of bacteria that used. Moreover, *P. aeruginosa* was the most affected than the rest, that shown in **Table 2** and **Figs. 3** and **4**.

Table 3. Antioxidant activities by DPPH radical scavenger activity of Standardized with respect to Ascorbic acid measurement. M ± SD (n = 6)

Samples	DPPH activity %
Ascorbic acid (0.4 mg/ml)	65.95± 8.78
Lycopene (0.4 mg/ml)	57±10.32
Ascorbic acid (0.8 mg/ml)	64.67±8.47
Lycopene (0.8 mg/ml)	59.33±7.50

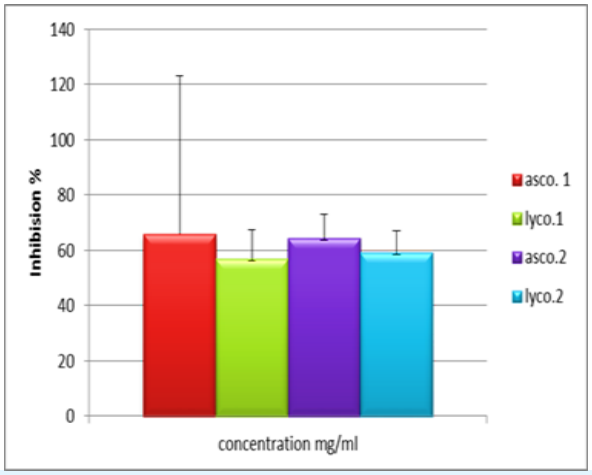

Fig. 5. Scavenging activity (%) on DPPH radicals of Lycopene extract and used ascorbic acid as a standard in two different concentration (0.4 and 0.8 mg/ml). All value is conveyed as M ± SD (n = 6)

Antioxidant activity by DPPH radical scavenging assay

The antioxidant action of the lycopene extraction was assessed via measuring, a free radical scavenger activity (DPPH).The two concentration of lycopene extract revealed non-significant ($p<0.05$) differences among samples as shown in **Table 3** and **Fig. 5**.

DISCUSSION

In this experiment used fifty different solvents system so give a wide range of choices to the best system must be used in addition to its availability. Also, the absorbance of whole samples that prepared have been used two times to read to know the influence of time on lycopene contented, in a second time (after 2 h). its effect was negative in mostly solvent systems which have been use. This perhaps due to the drop in the extract power of solvents with time furthermore less this result agreement with data of Dipen et al. (2017). An essential source of lycopene is *Lycopersicum*

esculentum also related handled food products, in which lycopene set up more than 60% of the carotenoids present. Predictable methods used for carotenoids extraction as of several diverse sources have used pure solvents such hexane, acetone, and ethanol. The extraction method which has been used, it was little cost and does not need comprehensive services for the preparation**,** separation and purification of lycopene. To check the purity of the lycopene the TLC was accomplished. Adjudicating from a preceding report (Britton 2008), the single spot with no extra carotenoids on TLC can be recognized as lycopene, also reliable lycopene have the equal Rf value as in Myong et al (2013). An ultimate isolation of pure lycopene from the extraction is in general accomplished by chromatographic methods HPLC evaluates were done using a Chromolith RP−C18E, 4.6 × 10 mm column on lycopene obtained by extraction from tomato with different mixtures of solvents. The presence of two peaks represented to lycopene and other to carotene, lycopene peak was irregular demonstrating the existence of cis-isomers and other carotenoid compounds, this result agreement with Saima (2014).

Lycopene can play antimicrobial role due to its antioxidant activity. Lycopene is unique of the public pigment extremely believed using diet manufacturing such as a food flavor moreover designed for its health incomes(Chandra et al. 2008).. In this current study, lycopene extract yields antimicrobial effect on some microorganisms especially *P. aeruginosa***,** the results acquired revealed antibacterial action is associated with the incidence active compound of lycopene extract. The antibacterial action of extract will contrast, when the source or the extract dilutes with other material; It is probable rises the efficiency of antibacterial than soluble in a single solvent. The microorganisms such as *Proteus, S. aureus, Bacillus*, also antifungal *Aspergillus niger* and *C. albicans* affected by Tomatoes (Sung et al. 2007). Previous study exhibited that the antimicrobial activity of tomato extract is due to existence of active constituents of tomato in extract on various bacteria (Al-Oqaili et al. 2014). Other study agreement with our result and that showed lycopene can exhibit effect on the growth of *P. aeruginosa* more than other bacteria (Ranjbar and Ranjbar 2016). The common usual metabolism of aerobic cells is related with free radical formation. The oxygen depletion intrinsic in growing of the cell leading to production of a chains of OFR. A communication of these types by way of particles of the fatty nature creates different radical such as OH plus diverse peroxides such as hydroxyl, super oxide and lipoid peroxides work together with biotic system in an obviously cytotoxic style (Aust and Sringen 1982). The antioxidant actions as resolute via the DPPH assay was greater in red tomatoes, the mechanisms of action of DPPH, is scavenge of DPPH cationic radical, a result of the assays in our study was non-significantly (P>0.05) associated between lycopene extract and ascorbic acid which is the standard used. The action of carotenoids amplified with aggregate the number of conjugated double bonds, they have 11 conjugated double bonds (Mullar et al. 2011). Lycopene has exclusive the structural as well as chemical structures that will give to its definite biological properties (Liu et al. 2008). The construction of lycopene is an acyclic hydrocarbon carotene per11 double bonds adding to was reflected to remain advanced capable quencher of single oxygen ($O_2^{\bullet}$) through β-carotene (Chen et al. 2007).

CONCLUSION

by systems of the solvents extraction method aimed at effectual extracted of lycopene as of tomato can be through using chloroform: methanol also CCl_4; in two different periods but in Benzene: Ethanol after only 2hr. As for the other method which have been used for lycopene extraction from tomatoes by simple solvent,it was successful and inexpensive that confirmed by results of TLC and HPLC. Also The results acquired from this study appearances that the lycopene extract have inhibitory potential in contrast to some of microbes,and most effective is *Pseudomonas aeruginosa*. This study revealed strong antioxidant activity of lycopene by DPPH radical scavenging activity which do not differences with ascorbic acid.

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