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Brief report

Antimicrobial Property of Lycopene Oleoresin on some Food Pathogens
Running Head: Lycopene oleoresin antibacterial potent

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Abstract

The aim of this work was to study the antimicrobial activity of tomato skin lycopene oleoresin against Pseudomonas aeruginosa ،*Escherichia coli* ،*Staphylococcus ureuse* ،*Salmonella typh* ، *L. monocytogenes* ، *Bacillus cereus* ،*Bacillus licheniformis*. Oleoresin was extracted from tomato peel. Lycopene content was measured by spectrophotometer. Lycopene oleoresin contained 2321mg lycopene/ 100 g oleoresin diluted in serial micro-dilution technique from 40,000 to 78.125 ppm. Microbial culturing was done in ELISA 96-well micro-titer plates in triplet and then MIC and MBC were determined. The results were shown that tomato peel oleoresin contained 2% lycopene, can inhibit and restrain the gram positive and negative bacteria.

Keywords: lycopene oleoresin, tomato skin, antimicrobial activity, ELISA, dilution method

Introduction

The medical properties of herbal extracts and essential oils against microbial [1, 2] and non-microbial [3, 4] diseases are known since ancient times .Many studies on different species of plant extracts or essential oils and their effects on microorganisms has been done. Antimicrobial properties of different plant extracts are reported [5, 6]. Antimicrobial effects of essential oils are reported on bacteria and yeasts more [5, 6] but fungi are also influenced by antimicrobial properties of essential oils [7, 8]. 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 [9].

Lycopene is dominant colorant in tomato, watermelon, papaya, and red pepper. The most important sources of lycopene is tomatoes. Depending on varieties they represent 65- 98% of total weight of carotenoids in tomato [10 and 16]. This pigment is a carotenoid with 11 conjugated double bands which besides the coloring ability, have antioxidant property and can inhibit the incidence of lung, prostate and stomach cancer. Today there are more trends in use of this natural and healthy component in human diet. Lycopene due to its antioxidant activity can play antimicrobial role. Lycopene is one of the popular pigments highly accepted by food industry as a food additive and also for its health benefits [17, 18]. As a red colorant and antioxidant agent, the demand for lycopene is still increasing. According to [17], total world consumption of lycopene was tripled to 15,000 tonnes in 2004 compared to 5000 tons in 1995.

Chandra *et al.* (2008) tested the effect of lycopene treatment in a randomized, double-blind study involving ten patients with clinical signs of gum inflammation. Half of the mount of each patient was also treated with oral prophylaxis. They found that lycopene treatment significantly decreased gum inflammation. The bleeding index of the gums treated with lycopene was further reduced by

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the oral prophylaxis. They also found that gum inflammation was strong negative correlated with uric acid level of the saliva [19]. Sung *et al.* (2007) also found that lycopene exerted potent antifungal activity on *Candida albicans* by causing significant damage to the cell membranes of the yeast. It showed little toxicity against human erythrocytes. More studies are required to demonstrate such effect in clinical experiment [20]. Al-Oqaili *et al.* (2014) studied the antibacterial activity of aqueous tomato extract on medical important pathogens. They found that the extract has inhibiting the growth of some isolated bacteria [21]. The objective of this study was analyzing the antimicrobial activity of tomato lycopene on Gram-positive and Gram-negative food-borne bacteria.

Materials and methods

Lycopene oleoresin preparation

At first ripened tomatoes immersed to boiling water for 1-2 minute and immediately cooled. Then peeled by hand. Skins were dried at 40^0C by convective oven. Extraction were carried out by hexane:ethanol:aceton (2:1:1) with 0.05% BHT as antioxidant by ratio of 1:10 (sample: solvent) by Sadler(1990) and Shi(1999) method at 30^0C with gently stirring [14, 15]. After 16 hr, the extracted solution was filtered by Watt man number 4 saturated with ethanol. 20% deionized water were added to filtrate and retained for 15 minute to divide into two phase. The upper phase is polar and contains lycopene and the under phase is aqueous and rejected. The upper phase is used for lycopene derivation [14, 15].

Lycopene quantity measurement

Spectrophotometer method was used to lycopene measurement [16, 22]. Lycopene absorption in hexane have three peak at 445 nm, 472 nm, and 503 nm which for reducing the intervention of other carotenoids and measuring total all-trans lycopene, quantification was carried out at 503 nm (λ_{Max}) in hexane (PG Instruments Ltd, UV/VIS T80) [16, 23]. The lycopene content in oleoresin, was calculated as below [23].

$$\text{Lycopene (mg)} = A \times dil \times ml \times 10 / E_{1cm}^{1\%} \quad (1)$$

wherein A is absorption in 1cm quvett; dil, dilution factor; ml, total volume of sample; and $E_{1cm}^{1\%}$, special extinction coefficient for lycopene in hexane (equals to 3450) [23].

Evaluation of antimicrobial activity

Antimicrobial activity of lycopene oleoresin was identified by determination of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) tests. Seven important pathogenic bacteria includes *Pseudomonas aeruginosa (*ISIRI 275*) ،Escherichia coli(PTCC 1533) ، Staphylococcus ureuse (*PTCC 1112*) ، Salmonella typhi (PTCC 1609) ، L. monocytogenes (PTCC 1163) ، Bacillus cereus* (ATCC 11778*) ،Bacillus licheniformis (*PTCC 1525*)* were analyzed triplicate by micro-dilution technique in ELISA 96-well micro-titer plate. With this method we determined MIC and then MBC of lycopene oleoresin. Microbial species were cultured on Müeller-Hinton Agar and incubated at 37^0C for 24 hr (Figure 1).

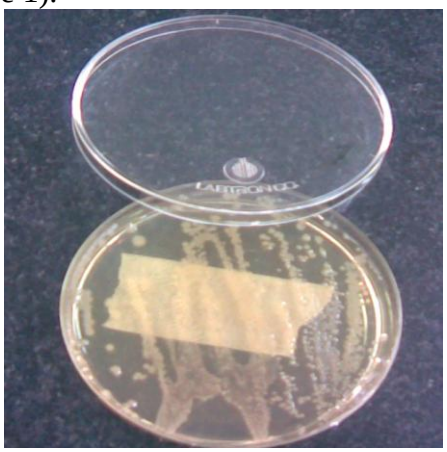

Fig.1. Bacteria cultured on Müeller-Hinton Agar

Microbial population equal to 10^6 CFU/ml from each bacterium was prepared at log-phase growth by adjusting the turbidity of population according to 0.5 McFarland standard. Bacterial inoculum was prepared by suspension of freshly grown bacteria in sterile saline (0.85% NaCl w/v). Double serial dilutions of lycopene oleoresin was prepared by DMSO with concentrations between 40,000 to 78.125 ppm. Medium without lycopene,

medium contained DMSO and different lycopene concentrations without addition of bacteria were used as control for each of mentioned components, respectively. After preparation of micro-titer plates, they were incubated at 37^0C for 18-24 hr (Figure 2)

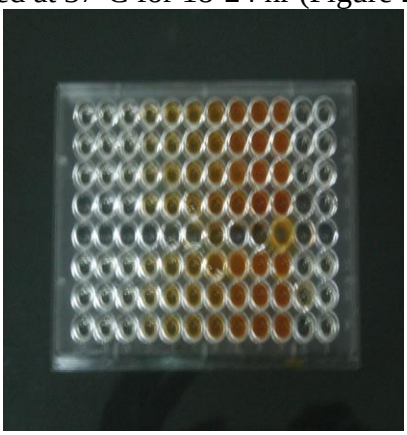

Fig. 2. ELISA 96-well micro-plate contained different concentrations of lycopene oleoresin

The turbidity were measured by ELISA READER (Bio-Tek Instruments) at 580 nm as a result of microbial growth and thus MIC were determined. MIC of lycopene oleoresin was determined by concentration with no visible bacterial growth. To detection of MBC, from wells without bacterial growth, point culturing was done at Nutrient Agar. After 24 hr incubation at 37^0C, the points without bacterial growth were reported as MBC.

Results and discussion

Oleoresin derived from tomato skin were extracted by solvents and its antimicrobial effect on *Pseudomonas aeruginosa* ، *Escherichia coli* ،*Staphylococcus ureuse* ، *Salmonella typhi* ، *L. monocytogenes* ، *Bacillus cereus* ،*Bacillus licheniformis* is shown in table 1. The results showed that lycopene oleoresin can inhibit and prevent the growth of studied bacteria. To prevent and inhibit the growth of Gram-positive bacteria, higher concentrations of lycopene oleoresin is needed with this evidence we can claim that rather than coloring and antioxidant ability of tomato lycopene oleoresin, it has very important property of antimicrobial activity on important food-borne bacteria. This can be a very important property for this component which increases its importance to use and application in food industry or pharmacy.

Table1- Antimicrobial effect of lycopene extracted from tomato peel on food-borne microorganisms

Bacteria	MIC (ppm)	MBC (ppm)
Escherichia coli (PTCC 1533)	2500	5000
L. monocytogenes (PTCC 1163)	20000	40000
Bacillus cereus (ATCC 11778)	1250	2500
Bacillus licheniformis (PTCC 1525)	2500	5000
Pseudomonas aeruginosa (ISIRI 275)	156.25	156.25
Salmonella typhi (PTCC 1609)	1250	2500

Al-Oqaili *et al.* (2014) showed that the antimicrobial activity of tomato extract is due to presence of active components of tomato in extract on some bacteria [21]. Dahan *et al.*2008 and Omodamiro *at al.* 2013 showed the effectiveness factor in tomato extract on bacteria, is lycopene that presence antimicrobial and antifungal activity [24, 25]. Lycopene oleoresin from tomato peel can exhibit the growth of *Pseudomonas aeruginosa* more than other bacteria. Both MIC and C are very low for this bactria. *But L. monocytogenes* was more sensible than others. The MIC of *L. monocytogenes* was 20000 ppm and the MBC was 40000 ppm. These amounts of MIC and MBC are high compared to other extracts. Mokami *et al.* (2014) showed the MIC and MBC of oils extracted from aerial parts of two medicinal plants, *Mentha longifolia* L. and *Rosmarinus officinalis* are lower than lycopene oleoresin. They found MIC and MBC of *Mentha longifolia* L. are 5000 and 5000 for *L. monocytogenes* and 10000 and 10000 for *Rosmarinus officials*, respectively [26]. They suggested this antibacterial power is due to oxygenated monoterpenes found in extracts.

Conclusions

This study revealed other than coloring and antioxidant activity, lycopene oleoresin can inhibit and prevent the growth of these very important studied bacteria in food. Today the demand for nature coloring agents is growing in developed countries, the lycopene extracted from tomato peel of food industries by-product, can play brilliant role in coloring,

antioxidant, and antibacterial activity. Thus we can look to lycopene rather than a coloring and antioxidant agent. Then we can recommit its use in diet as fresh using of its sources or its processed products and see its positive effects

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مقاله کوتاه پژوهشی

ویژگی ضدمیکروبی اولئورزین لیکوپن روی برخی پاتوژن‌های غذایی

عنوان کوتاه: توانایی ضدمیکروبی اولئورزین لیکوپن

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چکیده

هدف از انجام این تحقیق، مطالعه فعالیت ضد میکروبی اولئورزین لیکوپن پوست گوجه فرنگی در برابر *Pseudomonas aeruginosa*، *Escherichia coli*، *Staphylococcus ureuse*، *Salmonella typhi*، *L. monocytogenes*، *Bacillus cereus*، *Bacillus licheniformis* بود. الئورزین از پوست گوجه فرنگی استخراج شد. مقدار لیکوپن با اسپکتروفتومتر اندازه گیری شد. اولئورزین حاوی ۲۳۲۱ mg/۱۰۰g لیکوپن، به روش رقیق سازی میکرو، از غلظت ۴۰۰۰۰ ppm تا ۷۸/۱۲۵ ppm رقیق شد. کشت میکروبی در پلیتهای میکروتیتر ۹۶ خانه‌ای ELISA در سه تکرار انجام شد و سپس MIC و MBC تعیین شدند. نتایج نشان داد که اولئورزین پوست گوجه فرنگی حاوی ۲٪ لیکوپن، می تواند از رشد باکتریهای گرم مثبت و گرم منفی مورد مطالعه جلوگیری کند.

واژه‌های کلیدی: اولئورزین لیکوپن، پوست گوجه فرنگی، فعالیت ضد میکروبی، ELISA، روش رقیق سازی

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