

PHYTOCHEMICAL ANALYSIS OF POMEGRANATE PEEL EXTRACT (PPE) WITH THE EVALUATION OF ITS EFFICACY AS ANTI-RANCIDITY OF OILS, ANTI-BACTERIAL AND ANTI-CANDIDA AGENT

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ABSTRACT

Background: Pomegranate (*Punica granatum* L.) fruit is being cultivated since the civilization is known, and its production and consumption have been increased since the last century due to the scientific confirmation of its health benefits. The peels of pomegranate fruits are the major by-products enriched in antioxidants and broad-spectrum antimicrobial agents and can prevent food deterioration even.

Objective: To assess the quantitative assay for Alkaloids, Tannins, Phenolic acids, Saponins and Flavonoids in pomegranate peel extract (PPE), and evaluate the replacement of PPE as a natural anti-rancidity for corn in comparison with the widespread synthetic antioxidant TBHQ (28 ppm) with evaluation of the antibacterial and anti-candida effect of PPE.

Results: The quantitative assay for PPE were total flavonoids (304.21±0.43mg/g Rutin), total phenolic acids (352.60±0.54mg/g Gallic acid), total Tannins (1.66±0.11%), total Saponins (2.67±0.13 %) and total Alkaloids (1.51±0.17%). Using 28 parts per million of pomegranate peel extract and placing it on the oil increased the life of the oil and resisted rancidity for a period of 17.7 hr. compared to tertiary butyl hydroquinone TBHQ (28 ppm) which gave an approximate result of 19.68 hr. Based on the observed results, PPE was an active anti-bacterial and anti-candida substance for gram negative, gram-positive bacteria and candida. Diameter of the inhibitory area was about 20, 19, 23, 31, 17 and 18 mm against *K. pneumoniae*, *E. coli*, *S. aureus* and *S. haemolyticus*, *C. albicans* and *C. aures* respectively. *S. haemolyticus* was found to be more susceptible to PPE with an inhibition zone diameter of 31 mm. The MICs values against the bacterial and candida strains ranged from 250 µg/ml to 500 µg/ml, and minimum lethal concentrations (MLCs) ranged from 500 µg/ml to 1000 µg/ml

Conclusion: PPE is effective in food preservation, and has antimicrobial activities to provide a comprehensive guide for farmers, food processing, and storage sectors and academia.

Keywords: Pomegranate peel extract (PPE), anti-rancidity of oils, anti-bacterial, and anti-candida.

INTRODUCTION

Nowadays the natural antioxidants are cheap, environmentally friendly and sustainable resources has become main topic for the food industry and has been more preferred around the world by consumers than using toxic and synthesized antioxidants (Ibrahium, 2010; Padmaja & Prasad, 2011; Bopitiya & Madhujith, 2014 and Basiri, 2015). Pomegranate peel extract (PPE) has the capability to treat some chronic diseases due to its anticancer properties such as colon and prostate cancer, melanogenesis (skin cancer), breast cancer and stomach ulcers. In addition, pomegranate juice has the capability (as a powerful agent) to treat a variety of health problems such as Alzheimer's disease, asthma, prostate cancer, piles, diarrhea, stomach ache, coughing, sneezing, skin inflammation, piles, and hyperacidity (Hygreeva *et al.*, 2014; Basiri, 2015 and Derakhshan *et al.*, 2018). Furthermore, due to the excellent antioxidant activity of PPE, it has exhibited the potential activity as a cardiovascular protective agent inhibiting the formation and the accumulation of foam cells and cholesterol in the arteries (Hygreeva *et al.*, 2014 and Basiri, 2015).

Pomegranate peel potentially possesses higher amounts of polyphenolic content and antibacterial and antifungal activities suggesting its dual role as natural antioxidant and antimicrobial agent (Ibrahium, 2010; Wang *et al.*, 2011; Malviya *et al.*, 2014 and Rahnemoon *et al.*, 2016). It has been recently reported that the high amount of tannins such as punicalagin found in PPE

might be a key factor responsible for its antimicrobial activity (Ibrahium, 2010 and Rosas-Burgos *et al.*, 2017). Food industries have faced the challenge by looking for natural, safe, economic, and effective antioxidants originated from vegetables, fruits, plants, and agricultural-residues such as wheat and rice bran, peanut hulls, and old tea leaves (Konsoula, 2016). Vegetable oils originating from vegetable resources (such as sunflower, soybean, and corn) are high in polyunsaturated fatty acids and highly recommended globally as a human cooking media that have many beneficial and nutritional effects on the human body (Mei *et al.*, 2014 and Derakhshan *et al.*, 2018). PPE was noted to have antibacterial effects on different foodborne pathogens including *E. coli*, *F. sambucinum*, *P. italicum*, and *B. subtilis* (Tehraniifar *et al.*, 2011; Elsherbiny *et al.*, 2016 and Ismail 2016). Pomegranate peel was also tested as an incorporation agent into bio-based films and was found to improve the antibacterial activity of materials. In one of these studies, Ali *et al.* 2019 reported that PPE inhibited the growth of *S. aureus* (Gram-positive) and *Salmonella* (Gram-negative).

MATERIALS AND METHODS

Quantitative Analysis of Pomegranate peel extract (PPE): The quantitative assay is carried out for Alkaloids, Tannins, Phenolic acids, Saponins and Flavonoids.

Estimation of Total Phenolic Content (TPC): The amount of total phenolic in extract was determined with the Folin Ciocalteu reagent according to (Chun *et al.*, 2003 & Maurya and Singh 2010).

Estimation of Total Flavonoid Content (TFC): The amount of total flavonoid content in extract was determined by aluminum chloride assay through Colorimetric method as prescribed by (Samatha *et al.*, 2012 & Han and May 2012).

Estimation of Total Tannins using Gravimetric Method (Copper Acetate Method): This method depends on quantitative precipitation of tannin with copper acetate solution, igniting the copper tannate to copper oxide and weighing the residual copper oxide (Ali *et al.*, 2011). Estimation of Total Saponins: The saponins content was calculated in percentage according to (Obadoni and Ochuko 2001 and Okwu and Ukanwa 2007). The chloroform extract was filtered over anhydrous sodium sulfate and evaporated under reduced pressure till dryness, then weighed it to calculate the percent w/w. (Woo *et al.*, 1997).

Comparison of pomegranate peel extract against TBHQ 28.0 ppm: Natural antioxidant (methanolic extract) was added as PPE to Corn soft oil at concentrations (28.0 ppm) in comparison with both negative controls and positive

controls as recommended by previous studies (Mohdaly *et al.*, 2010).

Antibacterial and anti-Candida property: Tested microorganisms: Four isolates of bacteria *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus haemolyticus* as bacterial strains and *Candida albicans* and *Candida aures* as unicellular fungi isolated from clinical samples and identified according to (Bergey's manual 2009).

Agar well diffusion method: Pure cultures of the *K. pneumoniae*, *E. coli*, *S. aureus* and *S. haemolyticus* were spread on sterilized petri plates with Muller-Hinton agar. A circular well of 6 mm diameter was made in plates using a sterile cork-borer. The well was loaded with (100 µl) of PPE to check the antibacterial activity and the plates were incubated at 37 °C overnight and the zones were measured, on the other hand *C. albicans* and *C. aures* spread on sterilized petri plates with Potato Dextrose agar. A circular well of 6 mm diameter was made in plates using a sterile cork-borer. The well was loaded with (100 µl) of PPE to check the anti-Candida activity and the plates were incubated at 30 °C overnight and the zones were measured (Perez *et al.*, 1990).

Resazurin stain Preparation: The resazurin stain was prepared at 0.02% (Wt/Vol) (Khalifa *et al.*, 2013).

Determination of Minimum Inhibitory Concentration (MIC) for bacteria: The MIC of PPE was done using the method described in the guideline of (Cockerill 2010). On the other hand,

unicellular fungi were treated like bacteria as described above (Mekky *et al.*, 2021a).

Determination of Minimum Lethal Concentrations (MLCs): The MLCs of PPE against tested pathogens was assessed by the macro broth dilution assay

as described by Ansari *et al.*, with few modifications (Ansari *et al.*, 2015).

Statistical analysis: All the experiments were performed in triplicate and data were analyzed. Analyses were performed as prescribed by Kareem *et al.*, (2020).

RESULTS

Quantitative Phytochemical Analysis of pomegranate peel extract (PPE). The extraction efficiency of their phytochemical components (secondary metabolites) depends on some key factors such as the pomegranate part (leaves, peels, seeds, and flowers), pomegranate cultivars, solvent types, sample: solvent

ratio, extraction duration, pressure, and extraction temperature. In the present study we found that total flavonoids 304.21 ± 0.43 mg/gm rutin, total phenolic acids 352.60 ± 0.54 mg/gm Gallic acid, total Tannins $1.66 \pm 0.11\%$, total Saponins $2.67 \pm 0.13\%$ and total Alkaloids $1.51 \pm 0.17\%$ (fig.1).

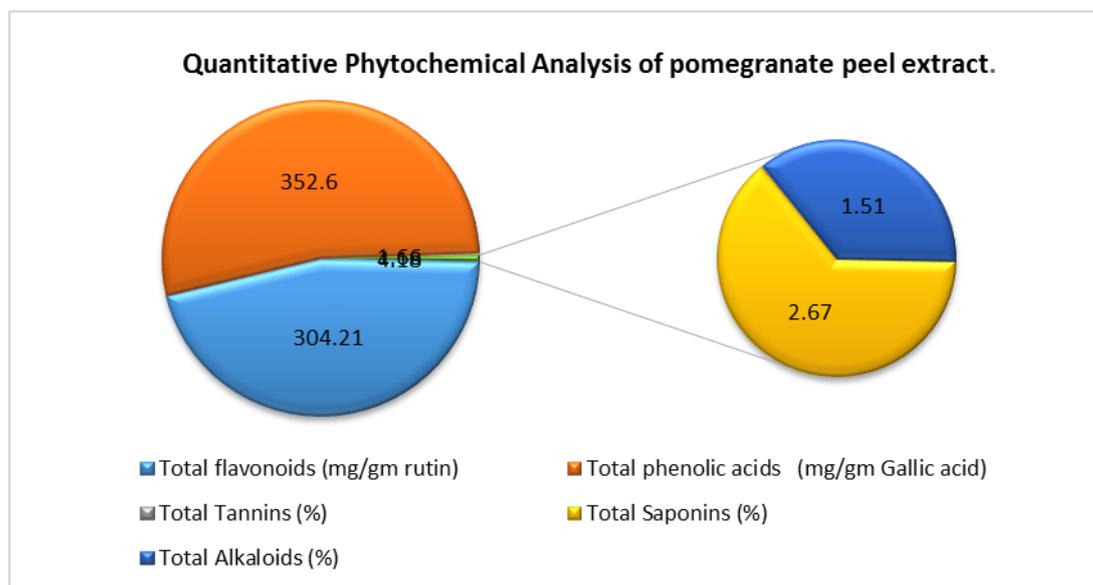


Fig. (1): Total active materials histogram of pomegranate peel extract (PPE).

Anti-rancidity of oils property of pomegranate peel extract (PPE): Since the beginning of the twentieth century, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butyl hydroquinone (TBHQ) have been widely used commercially as potential synthetic antioxidants mainly for oils due to their high content of polyunsaturated fatty

acids, The present study showed the importance of natural products especially PPE as anti-rancidity agent When using 28 parts per million of PPE and placing it on the oil increases the life of the oil and resists rancidity for a period of 17.7 hr. compared to tertiary butyl hydroquinone which gives an approximate result of 19.68 hr. (**fig. 2**).

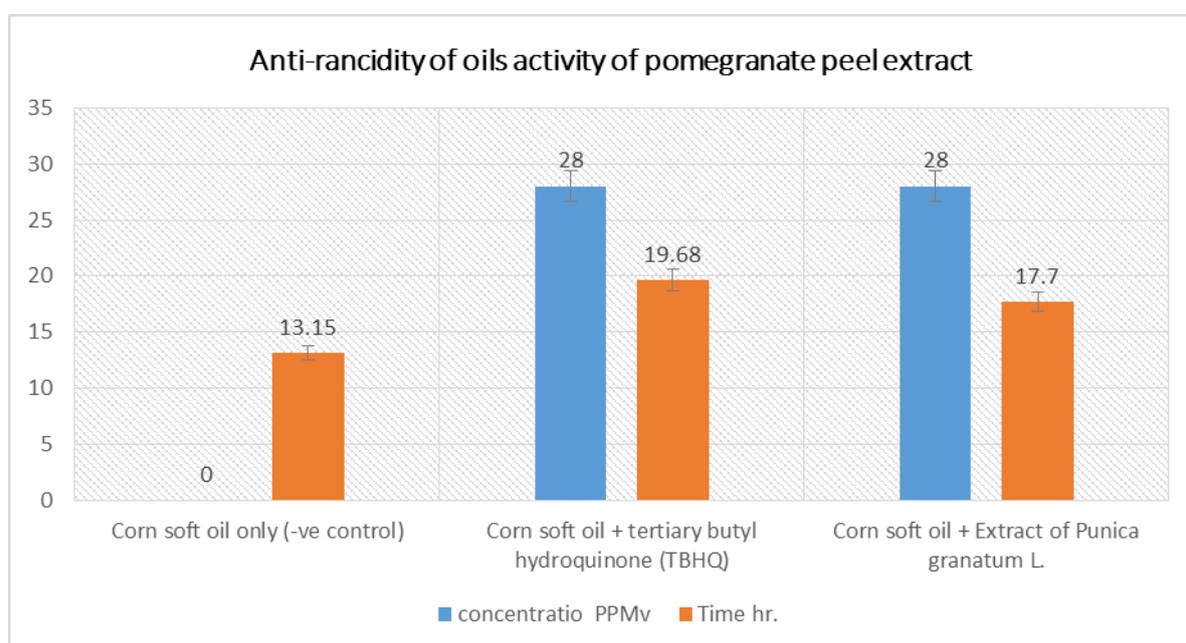


Fig. (2): Anti-rancidity of oils activity histogram of pomegranate peel extract (PPE).

Anti-bacterial and anti-candida property of pomegranate peel extract (PPE): Many studies showed that pomegranate extracts may be employed as natural alternative for the treatment of a wide range of bacterial infections due to their antimicrobial activity. Based on the observed results, PPE was an active anti-bacterial and anti-candida substance for gram negative, gram-positive bacteria and candida. In such cases, diameter of the inhibitory area is about 20, 19, 23, 31, 17

and 18 mm (**Fig. 3**), against *K. pneumoniae*, *E. coli*, *S. aureus* and *S.*

haemolyticus, *C. albicans* and *C. aures* respectively.

The result of the well diffusion method revealed that PPE inhibit the bacterial growth of the tested strains (*K. pneumoniae*, *E. coli*, *S. aureus*, *S. haemolyticus*, *C. albicans* and *C. aures*) at an appropriate volume of 100µl of PPE. The inhibition zones of the samples are compared with a pure medium as a

positive control and de-ionized water as a negative control, and the obtained zone indicates the antimicrobial activity of PPE. Based on the obtained results, *S.*

haemolyticus was found to be more susceptible to PPE with an inhibition zone diameter of 31 mm.

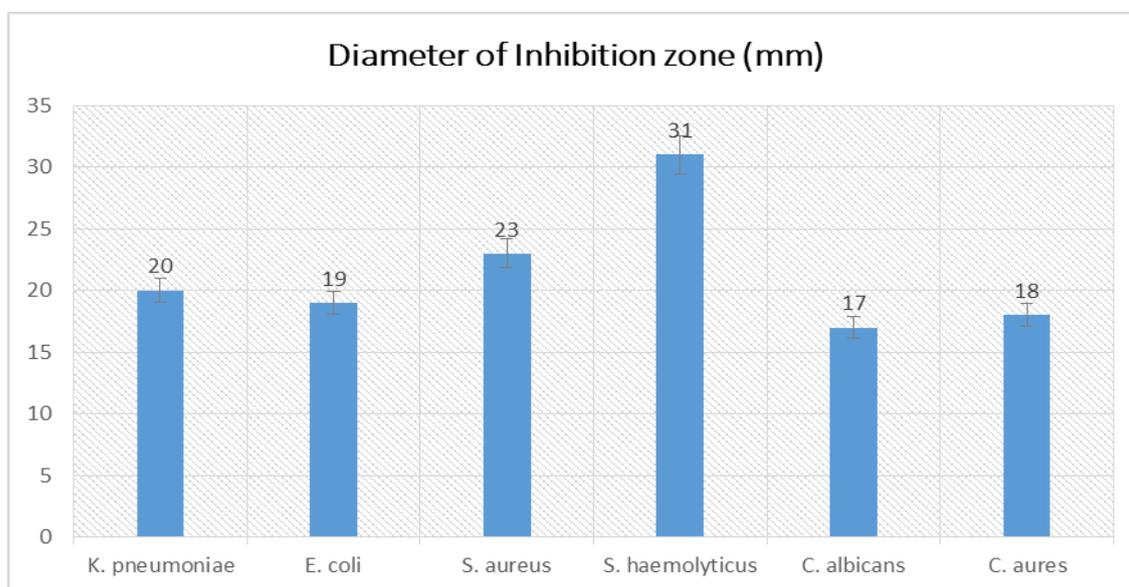


Fig. (3): Anti-bacterial and anti-candida activity histogram of pomegranate peel extract (PPE) (100 μ l) against bacterial and candida using inhibition zone diameter.

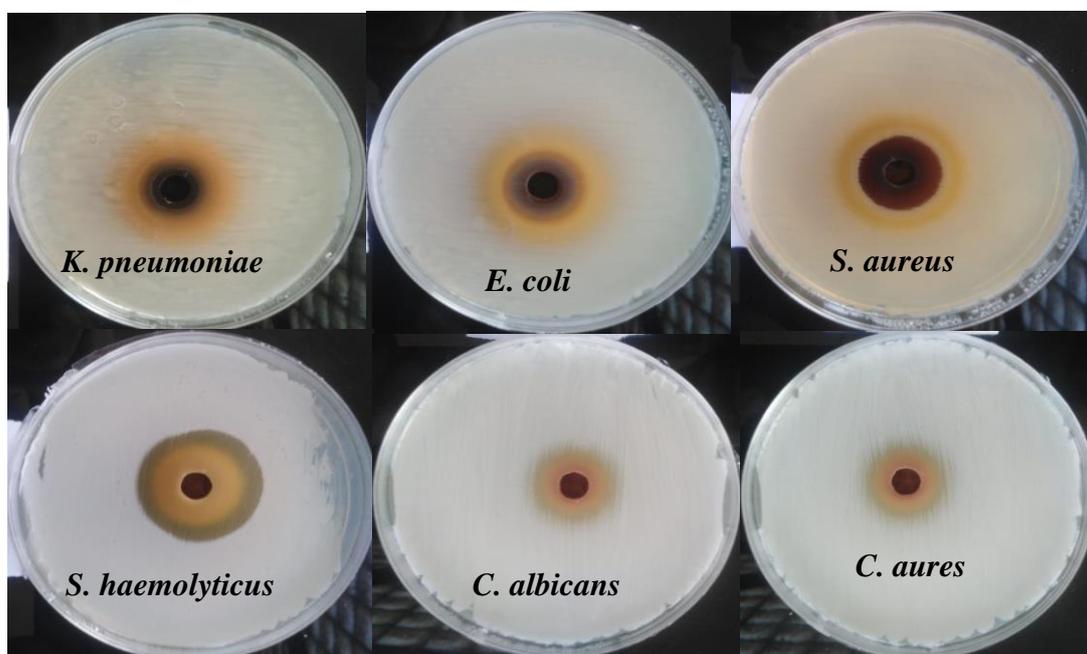


Fig (4): Inhibition zones produced against tested bacterial and candida strains: *K. pneumoniae*, *E. coli*, *S. aureus*, *S. haemolyticus*, *C. albicans* and *C. aures* using pomegranate peel extract (PPE) (100 μ l).

Determination of minimum inhibitory concentrations (MICs) and of minimum lethal concentrations (MLCs): The minimum inhibitory concentrations (MICs) values of PPE against the bacterial and candida strains ranged from 250 µg/ml to 500 µg/ml and minimum lethal concentrations (MLCs) ranged from 500 µg/ml to 1000 µg/ml see (fig. 5 and 6), *S. aureus* and *C. albicans* showed the MIC amount of 500 µg/mL. While remain strains showed MIC amount of 250 µg/ml.

Resazurin tincture was used in this study as an indicator to determine of microbial cell growth. Oxidoreductases

enzyme inside life microbial cells reducing the resazurin salt to resorufin and changing the blue non-fluorescent colour of resazurin salt to pink and fluorescent colour of resorufin See (Fig. 5 and 6). Many scholars showed that the antimicrobial activity of PPE was more potent than other parts, and the antimicrobial activity of PPE was related to the total flavonoids and tannins content. PPE is well known for its antimicrobial activity against bacterial and fungal pathogens

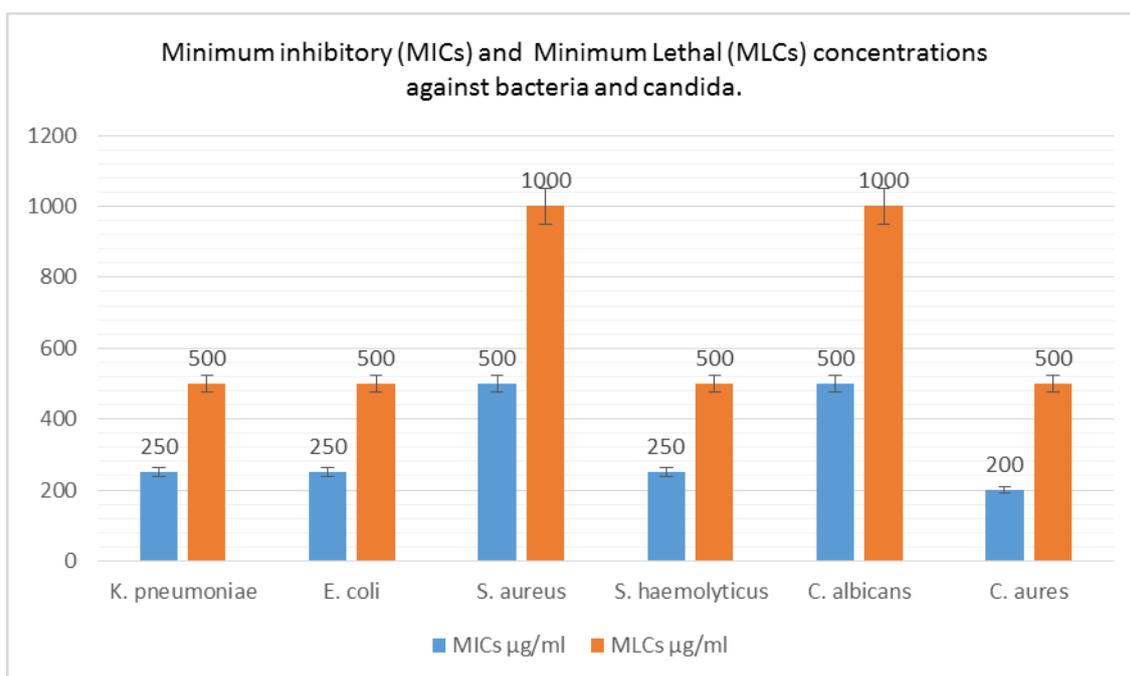


Fig. (5): Minimum inhibitory (MICs) and minimum Lethal (MLCs) concentrations histogram of pomegranate peel extract (PPE) against bacteria and candida strains.

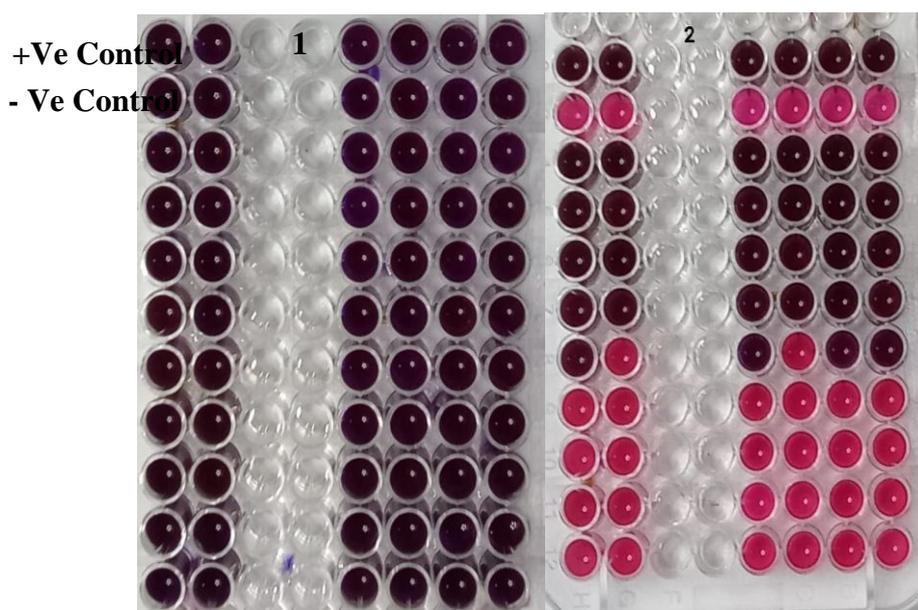


Fig. (6): Ninety-six well Microtiter plates of the colorimetric-XTT assay for determination of MICs values of pomegranate peel extract against bacterial strains using resazurin salt, (1) after addition resazurin dye and (2) the results after incubation

DISCUSSION

Punica granatum L. is widely known as “pomegranate” or “Paradise fruit” in many ancient cultures, and this fruit and all its derivatives (especially peels) are rich in most important secondary metabolites (phytochemicals) and have potential nutritional, economic and medicinal benefits in all over the world due to their wide range of important properties such as antibacterial, antioxidant, anti-atherosclerotic, anti-inflammatory and anti-allergic characteristics (Khalil *et al.*, 2018).

Among all the pomegranate parts, pomegranate peels have shown the maximum polyphenolic and flavonoid content followed by flowers, leaves, and seeds (Elfalleh *et al.*, 2012). In the present study, we found that total flavonoids 304.21 ± 0.43 mg/g rutin, total phenolic acids 352.60 ± 0.54 mg/gm Gallic acid, total Tannins $1.66 \pm 0.11\%$, total

Saponins $2.67 \pm 0.13\%$ and total Alkaloids $1.51 \pm 0.17\%$, in another study reported by Konsoula (2016), he found that PPE contained polyphenolics approximately three- to five fold higher than the existence of seed and juice extracts, respectively. Therefore, our findings were supported by previous results where methanol is the most preferable solvent for extraction of the majority of polyphenolic and flavonoid compounds from pomegranate samples (Padmaja & Prasad, 2011; Elfalleh *et al.*, 2012; and Konsoula, 2016).

Oily food products require a protective agent against auto-oxidation and chemical spoilage during storage (Ibrahim, 2010). Therefore, the addition of antioxidant agents in the food industry is highly required not only to preserve desirable taste, color, and flavor but also to overcome the stability problems and to increase the shelf life of oils and their derivatives (Mohdaly *et al.*, 2010).

The present study showed the importance of natural products especially PPE as anti-rancidity agent when using 28 parts per million of PPE and placing it on the oil increases the life of the oil and resists rancidity for a period of 17.7 hr. compared to tertiary butyl hydroquinone which gives an approximate result of 19.68 hr.

Food additives such as synthetic antioxidants have been the most applicable and effective methods to prevent oxidation, peroxidation, auto-oxidation of oily products, stabilizing oil characteristics, preventing oil rancidity, delaying oil deterioration and increasing the shelf life of lipids and lipid-containing products (Mohdaly et al., 2010, 2011 and Padmaja, Prasad 2011 and Konsoula, 2016).

Although synthetic antioxidants have significant capability in industrial practices to reduce chemical spoilage caused by oxidizing agents, previous researchers have confirmed the potential health risks and negative impacts of the long-term commercial use of synthetic antioxidants (Radhakrishna, 2014 and Mohdaly et al., 2011). Synthetic antioxidants might be responsible for the formation of peroxy and hydroxyl free radicals and other secondary toxic compounds, which might lead to serious human health concerns and might be associated with carcinogenic, mutagenic, and aging effects (Ibrahim, 2010 and Padmaja & Prasad, 2011).

The anti-bacterial and anti-candida action of PPE against bacterial strains has been determined on *K. pneumoniae*, *E. coli*, *S. aureus* and *S. haemolyticus* as

bacterial isolates in addition, the antifungal activity on *C. albicans* and *C. aures* as a candida isolates using well diffusion agar prescribed by other authors (Balouiri et al., 2016).

Based on the observed results, PPE was an active anti-bacterial and anti-candida substance for gram negative, gram-positive bacteria and candida. In such cases, diameter of the inhibitory area is about 20, 19, 23, 31, 17 and 18 mm against *K. pneumoniae*, *E. coli*, *S. aureus* and *S. haemolyticus*, *C. albicans* and *C. aures* respectively. Many studies showed that pomegranate extracts may be employed as natural alternative for the treatment of a wide range of bacterial infections due to their antimicrobial activity. Recent study showed that PPE have an effective antimicrobial activity, as evidenced by the inhibitory effect on the growth *K. pneumoniae*, *E. coli*, *S. aureus* and *S. haemolyticus*, *C. albicans* and *C. aures*. These results were consistent with the results informed by (Pagliarulo et al., 2016). In addition, experimental data strongly support the antibacterial activity of pomegranate extracts against oral pathogen (Subramaniam et al., 2012).

The minimum inhibitory concentrations (MICs) values of PPE against the bacterial and candida strains ranged from 250 µg/ml to 500 µg/ml and minimum lethal concentrations (MLCs) ranged from 500 µg/ml to 1000 µg/ml. *S. aureus* and *C. albicans* showed the MIC amount of 500 µg/mL. While remain strains showed MIC amount of 250 µg/ml. In another study the MIC values of pomegranate extracts determined in different studies significantly vary. For

example, the MIC against *S. aureus* isolates are reported to range from 0.62 to >250 µg/µl (Pagliarulo *et al.*, 2016).

Resazurin tincture was used in this study as an indicator to determine of microbial cell growth (McNicholl *et al.*, 2007). Oxidoreductases enzyme inside life microbial cells reducing the resazurin salt to resorufin and changing the blue non-fluorescent colour of resazurin salt to pink and fluorescent colour of resorufin (Mekky *et al.*, 2021b).

In a recent study (El-Khetabi *et al.* 2020) studied the in vitro and in vivo effects of aqueous PPE on the brown rot (caused by *M. laxa* and *M. fructigena*). They reported an inhibition varying from 76.65% to 90% on the control of mycelia growth. Intense fungicidal activity of PPE was then reported against *B. cinerea*, *P. digitatum*, and *P. expansum* by (Nicosia *et al.* 2016).

While the antibacterial activity of the pomegranate peel has been the subject of numerous researches, few studies have investigated the antibacterial activity of pomegranate juice against oral pathogens, such as *S. mutans* and *R. dentocariosa*. Kote and Nagesh (2011) conducted a clinical trial that showed the ability of pomegranate juice to reduce the microorganisms of dental plaque (*streptococci* and *lactobacilli*). Finally, this study demonstrate that PPE efficacy in food preservation, and antimicrobial activities.

CONCLUSION

PPE was an active anti-bacterial and anti-candida substance. Diameter of the inhibitory zones ranged from 17 to 31 mm against tested microorganisms.

The MICs values against tested microorganisms ranged from 250 µg/ml to 500 µg/ml and MLCs ranged from 500 µg/ml to 1000 µg/ml.

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التحليل الفيتوكيميائي لمستخلص قشر الرمان (PPE)

مع تقييم كفاءته كعامل مضاد لتزنخ الزيوت ومضاد للبكتيريا والكانديدا

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خلفية البحث تُزرع فاكهة الرمان منذ أن عرفت الحضارة ، وزاد إنتاجها واستهلاكها منذ القرن الماضي بسبب الإثبات العلمي لفوائدها الصحية وقشور ثمار الرمان هي المنتجات الثانوية الرئيسية المخصبة بمضادات الأكسدة والعوامل المضادة للميكروبات واسعة النطاق ويمكن أن تمنع تدهور الغذاء.

الهدف من البحث: تقييم الفيتوكيميائي للقلويدات والتانينات والأحماض الفينولية والصابونينات والفلافونويدات في مستخلص قشر الرمان وتقييم استبدال مستخلص قشر الرمان كمضاد طبيعي للتزنخ للذرة مقارنة بمضادات الأكسدة الاصطناعية واسعة الانتشار (ثلاثي بيوتيل هيدروكينون 28 جزء في المليون) مع تقييم التأثير المضاد للبكتيريا والكانديدا لمستخلص قشر الرمان.

نتائج البحث: أظهرت النتائج المتحصل عليها أن التقييم الفيتوكيميائي لمستخلص قشر الرمان كانت عبارة عن مركبات الفلافونويدات الكلية (0.43 ± 304.21 ملجم / جم روتين) ، والأحماض الفينولية الكلية (0.54 ± 352.60 ملجم / جم حمض الجاليك) ، والتانينات الكلية ($1.66 \pm 0.11\%$) ، وإجمالي الصابونينات ($2.67 \pm 0.13\%$) وإجمالي القلويدات ($1.51 \pm 0.17\%$). كما أظهرت الدراسة الحالية أهمية مستخلص قشر الرمان كعامل مضاد للتزنخ عند استخدام 28 جزء في المليون من مستخلص قشر الرمان ووضع على الزيت يزيد من عمر الزيت ويقاوم التزنخ لمدة 17.7 ساعة مقارنة بالثلاثي بيوتيل هيدروكينون (28 جزء في المليون) والذي يعطي نتيجة تقريبية بلغت 19.68 ساعة. ومستخلص قشر الرمان مادة فعالة مضادة للبكتيريا سالبة الجرام وموجبة الجرام والكانديدا. يبلغ قطر المنطقة المثبطة حوالي 20 و 19 و 23 و 31 و 17 و 18 ملم مقابل كلبيسيلا نيومونيا وايشيريشيا كولاي واستافيلوكوكس اوريس واستافيلوكوكس هيموليتيكس

وكانديدا البيكانس وكانديدا اروس على التوالي. كما كانت استافيلوكوكس هيمولينيكس أكثر حساسية لمستخلص قشر الرمان مع منطقة تثبيط قطرها 31 مم. وتراوحت قيم أقل التركيزات المثبطة للنمو من المستخلص ضد السلالات البكتيرية وكانديدا من 250 ميكروغرام / مل إلى 500 ميكروغرام / مل وتراوحت التركيزات المميئة من 500 ميكروغرام / مل إلى 1000 ميكروغرام / مل الاستنتاج هناك فعالية لمستخلص قشر الرمان في حفظ الأغذية وأنشطته المضادة للميكروبات التي تعطي دليل شامل للمزارعين وقطاعات تجهيز الأغذية والتخزين والأوساط الأكاديمية. الكلمات الدالة: مستخلص قشور الرمان وتزنخ الزيوت العوامل المضادة للبكتيريا وكانديدا.