STUDY THE SUSCEPTIBILITY OF ALCOHOLIC EXTRACTS OF SOME CITRUS FRUITS PEELS AS ANTIOXIDANTS AND ANTIMICROBIALS

Hadeer Abbas Niema Al-Hadrawi1, Laith Fareed Hasan Al-Obaidi2
1,2 Food Science Department- Faculty of Agriculture- University of Kufa

ABSTRACT

The current study included the alcoholic extraction of peels of some types of citrus fruits, which included lemons, grapefruit, and Mandarin, and studying their ability as natural antioxidants, by estimating the amount of phenols and flavonoids, and the antioxidant activity, in addition to studying the inhibitory activity of the studied alcoholic extracts. The results of the study indicated the superiority of the amount of phenols in the alcoholic extract of mandarin, which amounted to 272 mg/ml, followed by lemon peel extract of 245 mg/ml, while grapefruit recorded the lowest amount, which amounted to 238 mg/ml. As for the results of the amount of flavonoids in the alcoholic extract of lemon peel, it was the highest, reaching 41.73mg/ml followed by the extract of mandarin peels 21.44 mg/ml, and finally the alcoholic extract of grapefruit peels 20.1 mg/ml. As for the results of the antioxidant activity of the studied peels, represented by measuring DPPH, the results indicated that the extract of grapefruit peels had the highest antioxidant activity, reaching (16.35 µg equivalent ascorbic acid/ml) at a concentration of 25 mg/ml, followed by lemon peels, where the DPPH value reached (8.51 µg equivalent ascorbic acid/ml). And then mandarin peels (6.49 µg equivalent to ascorbic acid/ml) at the same concentration. As for the ability to bind ferrous ion, the mandarin peel extract recorded the highest binding rate, which reached (84.58%), followed by lemon peels (78.78%), and then grapefruit peels (76.18%). While lemon peel extract recorded the highest ability to Scavenging hydrogen peroxide, which reached (77.1%), followed by grapefruit peels, which amounted to (50.01%), while the mandarin peel extract recorded the lowest percentage (36.24%). As for the results of the reducing power, the extract of mandarin peels was the highest, as it reached (1.453nm), followed by the extract of grapefruit peels (1.177nm) and finally lemon peels (0.507nm). The results of the inhibitory ability of the alcoholic extracts of the citrus peels studied indicated that all the extracts possessed an inhibitory activity against E coli, Klebsella spp, and Staphylococcus aurus in different concentrations, where the inhibitory ability increased with the increase in the concentration of the extract used.

Key words: citrus peels, alcoholic extracts, natural antioxidants, inhibitory ability.

I. INTRODUCTION

Citrus is one of the largest and most popular commercially grown fruit crops around the world, and its importance is not only in economic terms, but it is also famous for its nutritional benefits for humans and animals, citrus fruits are available in several varieties that are consumed either fresh or processed, and after processing approximately 50% of the Fruit remains unused and is disposed of as waste which includes remnants of fruit pulp, peels and seeds and direct disposal of these wastes causes serious environmental problems, research and studies focus on developing better reuse to obtain an added value for phytochemicals as well as their safe disposal and these important chemicals obtained from citrus fruits include essential oils, flavonoids, citric acid, and pectin (Kavita and Neelima., 2018). The citrus processing industries produce huge amounts of waste annually, amounting to more than 40 million tons per year worldwide. The waste residue contains approximately 50% of the original mass of the fruit, of which 40-55% are peel, 30-35% inner tissue and 10% Seeds, as citrus waste mainly contains the peel and pulp residues and contains high amounts of soluble sugars, fibers and residual membranes from the inner peel, therefore, semi-solid waste is susceptible to bacterial growth and fermentation, in addition, the peels are rich in essential oils, especially limonene, which reaches (80-30)%, which has anti-bacterial properties and a number of value-added compounds of commercial importance can be extracted from citrus waste and used in the food and processing industries, pharmaceutical companies and home use, among these value-added active compounds in citrus fruits are mainly essential oils (D-limonen), flavonoids (Hesperidin, naringenin, neo-hesperidin), carotenoids
(lutein B-carotene, lycopene, zeaxanthin) and limonoids such as (limonin, normilin, limonoic acid), phenols (coumarin, phenolic acid, phloroglucinol), organic acids (citric acid, maleic acid, succinic acid), vitamins (ascorbic acid, niacin, riboflavin), carbohydrates, pectin, enzymes (phosphatase, peroxidase) (Sharma et al., 2017).

Citrus fruits are a good source of phenolic compounds that can be extracted and used as natural antioxidants to prevent the oxidation of certain foods, or they can be used in the manufacture of functional foods (Patil et al 2009; Albishi et al., 2013). The total antioxidant capacity of plant extracts is affected by their chemical composition and antioxidant content (Kumaran and Karunakaran 2006). Therefore, there is a need for natural antioxidants to be used in food and medical materials and to replace industrial derivatives with natural antioxidants (Ramesh et al., 2011).

II. MATERIALS AND METHODS

2.1 Citrus peels (lemon, grapefruit, and Mandarin)

Lemons (citrus limon), grapefruits (citrus paradis) and tangerines (citrus reticulata) of African origin were obtained from local markets, where the fruits were washed with distilled water to remove dirt, dust and foreign materials. Then they were peeled and the peels were taken and cut into small pieces and dried by spreading them in trays at room temperature away from sunlight. After making sure that they were completely dry, the peels were ground by a laboratory mill, sifted, placed in dark cans, and kept in the freezer (–18°C) until use.

2.2 Alcoholic Extraction of the citric Peels

Citrus peel samples (lemon, grapefruit and mandarin) were extracted according to the method described by (Elnastas et al., 2015) by soaking 100 g of crushed citrus peels in 500 ml of ethanol with a concentration of (98%), mix the mixture well using a magnetic stirrer, then leave the mixture for 24 hours at a laboratory temperature of 25°C, then the extracts were filtered with Whatman No 4 filter paper, then the resulting extracts were concentrated using a rotary evaporator at a temperature of 40°C. Then the concentrated extracts were left at the laboratory temperature (25°C) to dry, and then the resulting extracts were placed in dark containers and kept by freezing at a temperature (–18°C) until use.

2.3 Determination of the Total Phenols for alcoholic extracts of citrus peels

The total phenol content of the studied citrus extracts was estimated based on the method described by Ayoola et al., (2008). The amount of phenols was calculated depending on the relative relationship between acid concentration and absorption at a wavelength of 760 nm using the standard solution (Galic acid).

2.4 Determination of flavonoids for alcoholic extracts of citrus peels

The method mentioned in Ayool et al., (2008) was relied upon in estimating the content of total flavonoids. The amount of total flavonoids for the studied citrus peel extracts was calculated based on the graphic relationship between the concentration of the compound and the absorbance, as different concentrations of standard rutin were prepared (5-80 mg/ml).

2.5 DPPH Test

The method presented in Marinova,, (2011) was used to estimate the antioxidant activity of the studied peels extracts.

2.6 Chelating of Ferrous Ion for the citrus Peels Extract

The ferrous ion binding capacity of alcoholic extracts of citrus peels was estimated according to the method presented in (Rashad et al., 2015).

2.7 Scavenging Hydrogen Peroxide of the citrus Peels Extract

The method described by Ruch et al., (1989) was adopted to estimate the scavenging of hydrogen peroxide radicals of alcoholic extracts of citrus peels.
2.8 Determining of Reducing Power of the citrus peels extract

The reducing power of alcoholic extracts of citrus peels was measured using the method mentioned by Huang et al., (2004).

2.9 Inhibitory Ability of Citrus Peel Extracts Against Some Pathogenic Bacteria

To study the ability of citrus peel extracts to inhibit the growth of some types of pathogenic bacteria, three types of bacteria were selected, namely E. coli, Klebsiella, and Staphylococcus aurus. Three concentrations of peel extracts of lemon, grapefruit and mandarin were tested (50, 100, and 200) mg/ml (Mod, 2015). The culture medium (Muller Hinton) was prepared according to the company’s method, and it was poured into Petri dishes of 20-25 ml, and left until solidification. 0.1 ml of the test bacteria was transferred and the inoculation was distributed over the medium using an L-shaped glass rod. Then the dishes were incubated at a temperature of 37 °C for two hours. The plates were then exit from the incubator and the media was punctured with a diameter of 5 mm with a cork borer under aseptic conditions. 200 microliters of the prepared extracts with the above concentrations were placed in the pits, and these dishes were transferred to the refrigerator for less than two hours, then the dishes were incubated at a temperature of 37 °C for 48 hours. Then the diameter of the inhibition zone, if any, was measured by means of a ruler, including the hole.

2.10 Statistical Analysis

The complete random design was used to analyze the chemical composition, analyze the total content of phenols and flavonoids, and analyze the antioxidant activity (reducing power, ferrous ion binding capacity, hydrogen peroxide capture ability). The type of extract and factor (B) is the concentration (SAS., 2004).

III. RESULTS AND DISCUSSIONS

3.1 Determination of the total content of phenols in the alcoholic extract of citrus peels

The amount of phenols in lemon, grapefruit and mandarin peels that were extracted with ethanol was determined on the basis of gallic acid equivalent mg/ml. Figure (3-1) shows the total content of phenols in the studied citrus peels, as the statistical analysis indicated that there were significant differences in the amount of phenols between the peels of the studied citrus fruits. The highest amount of phenols in the alcoholic extract of mandarin peels, which amounted to 272 mg/ml compared to lemon and grapefruit peels, which amounted to 254.3 mg/ml, was 238.2 mg/ml, respectively. When comparing the findings of this study with other studies, it was found that this result differed with the findings of Kawther (2016), who indicated that the highest amount of phenols in lemon peels was 142.68 mg CAE/mg extract, followed by grapefruit peels, where it reached 59.68 mg CAE/mg extract and finally the mandarin, which amounted to 52.83 mg CAE/mg extract. Muhammad et al (2016) indicated that the amount of phenols for the mandarin peel extracted with ethanol concentration (100%) was 178.75 mg GAE/g extract, while the amount of phenols for the mandarin peel extracted with 80% ethanol was 371.16 mg GAE/g extract, and the amount of phenol extracted with 50% ethanol was 276.52 mg GAE/g extract. The reason for the difference in the amount of phenols in the studied citrus peels and what was found by the rest of the studies may be due to the difference in type, variety, cultivation conditions, extraction method, solvent type and concentration.

![LSD= 7.45](image-url)
3.2 Determination of the amount of flavonoids in citrus peels

Figure (3-2) indicates the amount of flavonoids present in citrus peels (lemon, grapefruit and mandarin), as the results of the statistical analysis showed significant differences in the amount of flavonoids between peels, as the highest amount of flavonoids in lemon peels reached 41.71 mg/ml, followed by mandarin peels 21.44 mg/ml and then grapefruit peels 20.1mg/ml. Zhang et al. (2018) indicated that the amount of flavonoids in mandarin peels was 38.97 mg RE/g, and in mandarin seeds 10.48 mg RE/g, while in mandarin juice, the amount was 31.32 mg RE/g.

Al-Haraki (2014) showed that the amount of flavonoids in lemon, grapefruit, mandarin and orange peels extracted with 80% methanol is (34.99 mg of rutin / g), (16.77 mg of rutin / g), (29.65 mg of rutin / g), (24.96 mg of rutin / g) respectively. Kawther (2016) mentioned that the highest percentage of flavonoids was in mandarin peels, which amounted to 19.3 mg CE/mg extract, followed by lemon peels, which amounted to 16.24mg CE/mg extract, and then grapefruit 15.17mg CE/mg extract. The reason for the difference in the amount of flavonoids in the studied citrus peels and what was found by the rest of the studies may be due to the different types and method of extraction and the type of solvent used.

Al-Haraki (2014) showed that the amount of flavonoids in lemon, grapefruit, mandarin and orange peels extracted with 80% methanol is (34.99 mg of rutin / g), (16.77 mg of rutin / g), (29.65 mg of rutin / g), (24.96 mg of rutin / g) respectively.

Kawther (2016) mentioned that the highest percentage of flavonoids was in mandarin peels, which amounted to 19.3 mg CE/mg extract, followed by lemon peels, which amounted to 16.24mg CE/mg extract, and then grapefruit 15.17mg CE/mg extract. The reason for the difference in the amount of flavonoids in the studied citrus peels and what was found by the rest of the studies may be due to the different types and method of extraction and the type of solvent used.

3.3 DPPH test for Alcoholic Citrus Peel Extract

The results of Table (3-1) showed the antioxidant activity of the DPPH system, as the statistical analysis showed that there were significant differences between the studied citrus peels, as well as significant differences between the studied concentrations and the interaction between the type of peels and the concentration. The results of the table indicated that the highest antioxidant activity was for the grapefruit peel extract, as it reached at a concentration of 10 mg/ml (9.62 µg of ascorbic acid equivalent /ml) and the effectiveness increased with an increase in concentration, reaching (16.35 µg of ascorbic acid equivalent/ml) at a concentration of 25 mg/ml followed by the alcoholic extract of lemon peels and then grapefruit peels, as it reached in concentration 10mg/ml (5.21, 3.60 µg ascorbic acid equivalent /ml) respectively, and the antioxidant activity increased significantly with increasing concentration for both types of peels, reaching (8.51 and 6.49 µg ascorbic acid equivalent /ml) respectively at a concentration of 25mg/ml. Nur., (2019) reported that the antioxidant activity of DPPH system of lemon peels was fresh lemon 1.30 mg/ ml, frozen lemon, 0.82 mg/ ml, fresh key lime 1.83 mg/ ml, frozen key lemon 1.57 mg/ ml, fresh musk lime 3.16 mg/ ml, frozen musk lime 2.70 mg/ ml. As for Hafiz et al (2020), it was shown that the antioxidant activity of the DPPH system of grapefruit peels is 9.17 mg AAE/g and orange peels 4.79 mg AAE/g.

The DPPH is widely used and is a common technique for evaluating the free radical removal activity of different plant extracts, DPPH is a stable free radical soluble in ethanol or methanol and the reduction of free radicals is determined by the decrease in absorbance at 715nm when the color of the DPPH test solution changes from purple to light yellow. (Kalpna et al. 2011) and return it to DPPH-H by means of antioxidants that give it an electron or a proton, thus removing free radicals (Brand et al., 1995).

Table (3-1) DPPH test results for alcoholic extracts of citrus peels
### 3.4 Ability of alcoholic extracts of citrus peels to bind ferrous ion

Figure (3-3) shows the ability of alcoholic extracts of citrus peels to bind the ferrous ion and compare it with citric acid. The results of the statistical analysis showed that there were significant differences between citrus peels in their ability to bind the ferrous ion, where the citric acid was superior in their ability to bind the ferrous ion (89%) and it was followed by mandarin peels (84.58%) and then lemon peels (78.78%). While grapefruit peels recorded the lowest ability to bind the ferrous ion and reached (76.18%). The results of this study with regard to lemon peels and citric acid were close to what was found by Al-Qutaifi.,(2019), as the ability to bind the ferrous ion to lemon peels extracted with ethanol (100%) was 81.81%, while citric acid was able to bind the ferrous ion to 89.68%.

Geeth et al (2011) indicated that citrus extracts contain high amounts of active compounds and these active compounds have the ability to bind the ferrous ion and thus prevent oxidation, as the plant extracts have a chelating property that binds oxidation-stimulating metals through this prevents the formation of free radicals. As well as ascorbic acid, proteins, amino acids and peptides bind oxidation-stimulating minerals, so the ability to bind the ferrous ion in plant extracts is due to phenolic and non-phenolic compounds.

<table>
<thead>
<tr>
<th>Type of peel extract</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg/ml</td>
</tr>
<tr>
<td>Lemon</td>
<td>5.21</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>62.99</td>
</tr>
<tr>
<td>Mandarin</td>
<td>3.60</td>
</tr>
</tbody>
</table>

LSD for peels type = 0.649, LSD for the concentration = 0.309, LSD for the two ways interaction = 1.124

### 3.5 Ability of Alcoholic Extracts of Citrus Peels to Scavenging Hydrogen Peroxide

Figure (3-4) shows the ability of the alcoholic extracts of the studied citrus peels to Scavenging hydrogen peroxide and compare it with ascorbic acid. The results of the statistical analysis showed that there were significant differences between the studied peels in their ability to Scavenging hydrogen peroxide, where the figure showed that the highest ability to Scavenging hydrogen peroxide was for ascorbic acid, which amounted to 80%, followed by lemon peels (77.1%), then grapefruit peels (50.01%) and finally mandarin peels (36.24%). Al-Qutaifi (2019) indicated that the ability to Scavenging hydrogen peroxide for the ethanolic extract of lemon peels was 72.85%, and for ascorbic acid 85.71%. As for Abhishek et al (2011), they indicated the ability to Scavenging hydrogen peroxide for 85% of mandarin peels and 50% of grapefruit peels. Extracts of citrus peels contain high amounts of active compounds, and these active compounds have the ability to inhibit hydrogen peroxide, as hydrogen peroxide has the ability to react with Cu₂ and Fe² and the formation of hydroxide radicals, as these radicals have harmful effects on human health, so it is very important for cells to control the amount of hydrogen peroxide (Thampi and jeyadoss, 2015).
3.6 Reducing Power of Alcoholic Extracts of Citrus Peels

Figure (3-5) shows the reducing power of citrus peels (lemon, grapefruit, and mandarin), as the results of the statistical analysis showed that there were significant differences in the reducing power between citrus peels and ascorbic acid. The reducing power of ascorbic acid was 1.967 nm, which is the highest compared to the studied peels, followed by the reducing power of mandarin peels, which amounted to 1.453 nm, followed by grapefruit peels (1.177 nm), while lemon peels recorded the lowest reducing power compared to the rest of the peels, which amounted to 0.507 nm. Al-Qutaifi (2019) indicated that the reducing power of lemon extract was 2.041 nm and for the ascorbic acid was 2.163 nm.

Al-Halafi., (2016) indicated that the extracts that possess effective compounds have a high reducing power and have a better ability to give an electron and this electron will interact with free radicals and turn them into a more stable state as the reducing power gives an indication of the antioxidant activity and the reducing power is measured by a complex reduction ferricyanid converting Fe$_3^+$ to Fe$_2^+$ as the antioxidants present in the extracts have the ability to convert Fe$_3^+$ to Fe$_2^+$ and this can be known by forming a green or yellow dye at 700nm wavelength.

3.7 Inhibitory Activity of Alcoholic Extracts of Citrus Peels

The antimicrobial activity of the alcoholic extract of citrus peels with different concentrations was studied against *E. coli, Klebsiella spp.*, and *staphylococcus aurus* bacteria. Table (3-2) showed that the inhibitory activity of alcoholic extracts of citrus peels (lemon, grapefruit and mandarin) was close to all types of bacteria studied, and the results of the same table showed that with an increase in the concentration of the extract, the inhibitory ability increased.
of all types of peels studied increased. The inhibition diameter of the alcoholic extract of lemon peels at the concentration of 50mg/ml towards *E-coil*, *Klebsiella spp*, and *Staphylococcus aurus* was (13, 14, 15) mm, respectively and the diameter of the inhibition increased by increasing the concentration of the extract to 200 mg/ml, where the diameter of the inhibition reached (18, 15, 19) mm, respectively. As for the alcoholic extract of grapefruit peels, the inhibition diameter at the concentration of 50mg/ml towards *E-coil*, *Klebsiella spp*, and *Staphylococcus aurus* was (13, 12, 14) mm, respectively and the diameter of the inhibition was increased by increasing the concentration of the extract to 200 mg/ml, where the diameter of the inhibition reached (15, 16, 15) mm, respectively. While the inhibition diameter of the alcoholic extract of mandarin peel at the concentration was 50mg/ml towards *E-coil*, *Klebsiella spp*, and *Staphylococcus aurus* was (13, 12, 14) mm, respectively and the diameter of the inhibition increased by increasing the concentration of the extract to 200 mg/ml, where the diameter of the inhibition reached (18, 16, 19) mm, respectively.

Muhammad and Muhamad (2019) clarified that the inhibitory diameter of lemon peel extract against *E coli* bacteria was 15 mm, as for orange peel extract, the diameter of inhibition was 18 mm, and banana peels were 9 mm. Alhoi et al., (2018) indicated that the inhibitory diameter of lemon peel extract at a concentration of 25% was 15 mm, and the inhibitory diameter increased when the concentration was increased to 50%, reaching 16.17 mm, and it also increased at the 100% concentration, where the inhibitory diameter reached 18.77 mm. Also, Muhammad and Muhamad (2019) studied the effect of fruit peel extracts on *Klebsella pneumoniae* bacteria and indicated that the inhibitory diameter of orange peels was 12 mm, lemon peels 17 mm, and banana peels 12 mm. While Effiom et al (2019) showed that the inhibitory diameter of lemon peels against *Klebsella spp* at 20% concentration is 1 mm, while at concentrations (30 and 40) it was 1.5 mm, while the inhibitory diameter of mandarin peel against the same bacteria at concentrations 10% and 20% was 2 mm, while the inhibitory diameter increased when the concentration was increased to 30%, reaching 3 mm, and it increased more when the concentration was 40%, reaching 5 mm. The same study indicated that the inhibitory diameter of orange peels against *Staphylococcus aurus* was 13 mm, but for lemon peel extract, the inhibitory diameter was 13 mm, while the inhibitory diameter of banana peels was 12 mm. Citrus peels contain essential oils, and the essential oil includes limonene and γ-terpinen, which have an antimicrobial effect as they can disrupt the bacterial membrane and prevent respiration and ion transport (Moosavy et al., 2017). Therefore, when the membrane permeability is increased, essential oils pass through the cell wall and the cytoplasmic membrane may also coagulate in the cytoplasm and damage the lipid and protein layers (Moosavy et al., 2017). Flavonoids also have antimicrobial, antioxidant and anti-inflammatory activities that have been shown to inhibit specific enzymes, stimulate certain hormones and scavenge free radicals (Ezeabara et al., 2014).

**In Conclusion**, we can conclude that alcoholic citric peels extract which represented in this study lemon, grapefruit and mandarin have a good antioxidant capacity and a good ability to inhibit the growth of some pathogenic bacteria (*E coil*, *Klebsiella spp*, and *staphylococcus aurus*) and we recommended to use these extract to increase the shelf life of some food products such as meat, poultry and dairy products.

<table>
<thead>
<tr>
<th>Type of Alcoholic Extract</th>
<th>Concentrations</th>
<th>Inhibition Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>Lemon</td>
<td>50mg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>100mg/ml</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>200mg/ml</td>
<td>18</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>50mg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>100mg/ml</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>200mg/ml</td>
<td>15</td>
</tr>
<tr>
<td>Mandarin</td>
<td>50mg/ml</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>100mg/ml</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>200mg/ml</td>
<td>18</td>
</tr>
</tbody>
</table>
REFERENCES

1. Al-Qutaifi, Haider Kateh. 2019. Effect of adding powdered potato, onion and lemon peels on reducing the phytochemical properties of frozen and frozen beef tenderloins and diagnosing their active compounds. MSc thesis., College of Agriculture, University of Basra. Iraq.


19. Mod, Salia. 2015. Extraction of phenolic compounds from orange peels and studying their biological activity. Masters degree. Faculty of exact sciences. master degree university of martyr Hama AL Akhdar


