**Research Article**

**Evaluation of Antioxidant Activity of fresh Lemon (Citrus lemon) peel in Marrakech and Kenitra cities Morocco and Yemen**

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**Abstract**

The current study on fresh lemon peels to know the antioxidant activity and the content of polyphenols and flavonoids from regions located in Morocco, Marrakech and keneitra and a region Taiz in Yemen.

The test of polyphenol was performed from extracts that were measured using the Folin-Ciocalteu technique, also a test of Flavonoid was performed of was measured by using the aluminum chloride . The Free radical scavenging activity of citrus limon extracts was measured by 2,2′-Diphenyl-1-picrylhydrazyl hydrate (DPPH).

The DPPH radical scavenging activity of ethanolic extract of fresh Citrus limon peel was found to be highest at 200μl concentration which were 77, 15 %; 67, 88 %; 37,74%. Through it 50% inhibition (IC50) was obtained to be 92, 04; 153, 55 and 215,407 μg/ml in Marrakech, Kenitra, and Taize respectively. As well, the values radical scavenging activity of methanolic extract of the same concentration which are 82, 77 %; 53, 33 % and 47,5%. Through it 50% inhibition (IC50) was obtained to be 145, 6; 179, 17 and 274,899 μg/ml in Marrakech, Kenitra, and Taize respectively. But regarding, the values radical scavenging activity of essential oil of the same concentration which are 63,77 %; 42,19 % and 80,25%. Through it 50% inhibition (IC50) was obtained to be 155,54; 271,158 and 101,31 μg/ml in Marrakech, Kenitra, and Taize respectively.According to the results obtained in our study, the antioxidant activity increases with increased polyphenols and IC50 deficiency in Marrakech, Kenitra, and Taize

**Key words:** Citrus Limon – antioxidant activity- polyphenol – flavonoid - radical scavenging activity.

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**Competing Interest:** The authors have declared that no competing interest exists.

**Acknowledgment**

I thank all my family who support me, my father, my Mother, my brothers, sisters and all my friends. Special thanks to a Doctor Khedid Khadija about her a large effort with me to achieve this humble research.

**Introduction**

Citrus fruits are classified as one of the most productive agricultural crops in the world, such as orange, lemon, and mandarin R. Kummer et al., 20151; with an annual production of 135.9 million tons FAO, 20192 . The fruit peel contains secretary cavities filled with essential oil S. S. Voo et al., 20123. The extraction of essential oils from Citrus fruits dates back to the sixteenth century. Limonene is one of the most frequent and inexpensive fragrances used in cosmetics formulation and can be found in many types of beauty products such as soaps, perfumes, shampoos, hair conditioners, and shower gels. A. F. Filipsson,R et al.,19984; Hirota et al ., 20105 Citrus peels contain important chemical compounds that have value in the area of lunch and health. These include phenolic compounds, flavonoids, essential oils, vitamins and minerals Del Rıo et al6., 2004 ;Gil-Izquierdo et al., 20047. It is well established that phenolic and secondary metabolites with conjugated double bonds usually show substantial antioxidative properties Koh K et al ., 20028.And from Previous studies, citrus fruits have an effective role in preventing chronic diseases, diabetes, blood pressure, and some types of cancer (Zou et al., 2016)9.The current study on fresh lemon peels to know the oxidation activity and the content of polyphenols and flavonoids from regions located in Morocco, Marrakech and kenitra and a region in Yemen in Taiz.

**Materiel and Methods**

**Plant material:** The fruits of citrus were harvested at the beginning of the harvest in November 2019 from Morocco (Marrakech, Kenitra ) and Yemen (Taiz). The collected material was studied in the Regional Center for Agricultural Research in Kenitra, Marrakech. The study was in the Department of Drug Sciences, Laboratory of Medicinal Chemistry, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco.

**Preparation of Essential Oil**

The essential oils was prepared from fresh lemon (citrus limon) peel were prepared in Morocco ( Marrakech and Kenitra cities) and Yemen Taiz city by steam distillation (**Owosini et al**., 2015)10 ,We added 700 ml of distilled water to 200.22 g,200.24 g and 188,60 of fresh lemon (Citrus limon ) peel in Morocco (Marrakech, Kenitra) and Taiz City in Yemen respectively. The mixture was preheated for 6 hours at 4°C. Then, the yield was obtained by the relation between the mass of lemon peels and the mass of the essential oil

**Preparation of extracts**

The extracts was prepared from the fresh lemon (Citrus limon ) peels in Morocco (Marrakech, Kenitra) (62.85 g,65.05 g) and The fresh lemon (Citrus limon) peels in yemen Taiz city ( 62.5 g). We added 600 ml of each solvent individually by employing the Soxhlet. It was heated for 5 h at a temperature 4C of the according to the study of Lin et al, (1999) (Ewansiha JU et al2016) 11. Our solvents are Ethanol and Methanol, The filtration of the extract was made and concentrated to dryness. The yield of the extract obtained was calculated as follows: R (%) = M 1/ M2 × 100 Where: R: yield of essential oil M1: the mass of the essential oil obtained in g M2: the mass of Lemon peels in g.

**The chemical composition offresh Citruslimon peel**

The essential oil of fresh citrus limon peel was an Analysed of by employing gas chromatography-mass spectrometry (GC-MS) along with the mass spectrometer (Q-8 MS ion trap) by application of Adams data12.

**Determination of total polyphenol content**

 The polyphenol was assessed of C.limon peels by the method the Folin-Ciocalteu according to ( Lister and Wilson ., 2001) 13. 0,5 mL were accoutred from the ethanolic extracts and methanolic extracts in Morocco exactly from Marrakech, Kenitra cities and from Taiz city in Yemen, we added 4 mL of sodium carbonate (7.5 %, w/v) and added 2,5 mL of FolinCiocalteu reagent, and also dilute it with distilled water by 1:10 ratio. Then the samples were incubated at 45 C for 30 minutes for the development of a blue color. The absorbance measurements were made at 765nm using a UV-Vis spectrophotometer in comparison with the blank solution. Under the same conditions, the standard curve of Gallic acid was obtained over a concentration range of 0-500 (μg/ml). The values of phenolic contents were expressed as Gallic acid equivalent (mg GAE/g extract). The total polyphenol contents in all samples was calculated the using the formula: C = c V/m where, C = total polyphenol content mg GAE/g dry extract, c = concentration of gallic acid obtained from calibration curve in mg/mL, V = volume of extract in ml, m = mass of extract in gram Statistical analysis.

**Determination of Total Flavonoid content**

The test Flavonoid's was performed of ethanolic and methanolic extracts of the fresh Citrus limon peels in Marrakech and kenitra Morocco cities and in yemen Taiz city by emplyement of the aluminum chloride colorimetric according to (Ordon et al ., 2006)14.0,5 mL of each sample was mixed with 0,5 mL of 0,2 aluminum chloride 10%. Diluted with methanol to a ratio of 96%, 0,2 ml potassium acetate and 5 ml of water distilled . also the mixture was incubated at a temperature 40 C for 30 min. The absorbance measurements were synthetic, at 420 nm by utilzing a -Vis spectrophotometer in comparison with the blank solution. beneath the same conditions, the standard curve of quercetin was gained over a concentration range of 0- 100 (μg/ml). The values of flavonoid contents were expressed as quercetin equivalent (mg GAE/g extract).

**Antioxidant Activity (AA)**

 The Free radical scavenging activity of citrus limon extracts was measured by 2,2′-Diphenyl-1-picrylhydrazyl [hydrate](https://www.sciencedirect.com/topics/chemistry/hydrate)  (DPPH) ( Huang et al ., 2011)15. Thus, (0.2 mM) was prepared by dissolving 7.8 mg of DPPH in 100 mL methanol at room temperature for 2 h in a dark place to complete the reaction. The different concentrations (20,40, 60,80,120,140,160,180 and 200 µg/ml) of solutions of each extract were prepared by the serial dilution of the stock solution (4 mg/ml) of citrus Limon peels extract. To each 0.5 ml extract solution, 2.5 ml of DPPH solution was added. A control was prepared by mixing 0.5 ml distilled water and 2.5 ml 0.1 mM DPPH solution. These samples were shaken well and kept in dark for 30 minutes at room temperature. The absorbance was measured at 517 nm against the blank solution consisting of 2.5 ml MeOH and 0.5 ml distilled water. Percentage of DPPH Scavenging Activity determined as follows% DPPH radical scavenging = [(absorbance of control – absorbance of the test sample) ÷ (absorbance of control)] × 100.

 **Results and discussion**

**The yield of fresh lemon peel extracts**

The fruits of citrus were harvested at the beginning of the season in November 2019. Then, we got the essential oil extraction HE of fresh citrus limon peel by steam distillation using a Clevenger. Also, we got the extracted methanol EM, and the extracted ethanol EE by using Soxhlet. The yield rate of essential oil HE, methanol extract EM, and ethanol extract EE were 0.78%; 9.8%; 10.05%, 0.64%, 8.3%, 8.9 % and 0,90 %; 8,8%,8,6% in Marrakech, Kenitra, Taize respectively Table1.

**Table 1: The yield rate of fresh Citrus limon peel in Marrkech, Kenitra and Taize**

|  |  |  |  |
| --- | --- | --- | --- |
| Extract |  Marrakech | Kenitra | Taize |
| HE | EM | EE | HE | EM | EE | HE | EM | EE |
|  YieldR % | 0.78 | 9.8 | 10.05 | 0.64 | 8.3 | 8.9 | 0,90 | 8,2 | 8,6 |

Key: *HE:essential oil,EE: ethanol extract, EM :methanol extract*

It is clear from the results obtained in Table 1. The yield rate of essential oil was 0,90% in Yemen higher than the yield in the Marrakech was 0.78% in Marrakech higher than the yield in the Kenitra was 6.58 %. While the yield rate of extracted methanolic (EM) and extracted ethanolic (EE) was 9.8% and 10.05% in Marrakech higher than the yield in the Kenitra was 8.3%, and 8.9% in Keniter a higher than the yield in the taize was 8,2% and 8,6%respectively. Therefore, the yield ratio of extraction ethanol is higher than that of extraction methanol and essential oils.

**Table 2:Chemical compounds of essential oil of fresh Citrus limon peel**

**that is grown in Morocco(Marrakech, Kenitra ) and Tize**

|  |  |  |  |
| --- | --- | --- | --- |
| **Chemical compounds** | Marrakech | Kenitra | Taize |
|  Citrus limon HE |  Citrus limon HE | Citrus limon HE |
|  P % |  P % |  P % |
| β-Myrcene |

|  |  |
| --- | --- |
| 1,20 |  |

 |

|  |  |
| --- | --- |
| 2,67 |  |

 | 0,14 |
| D-Limonene |

|  |  |
| --- | --- |
| 39,49 |  |

 |

|  |  |  |
| --- | --- | --- |
| 29,19

|  |
| --- |
|  |

 |  |

 | 9,41 |
| Linalyl Acetate |  14,07 |

|  |  |
| --- | --- |
| 14,54 |  |

 | 3,86 |
| α-Terpineol |

|  |  |
| --- | --- |
| 4,90 |  |

 |

|  |  |
| --- | --- |
| 10,47 |  |

 | 9,40 |
| α-Pinene |

|  |  |
| --- | --- |
| 7,93 |  |

 |

|  |  |
| --- | --- |
| 0,64 |  |

 | 0,11 |
| β-Pinene |

|  |  |
| --- | --- |
| 5,54 |  |

 |

|  |  |
| --- | --- |
| 4,69 |  |

 | 0,86 |
| Carvacrol |

|  |  |
| --- | --- |
| 1,25 |  |

 |

|  |  |
| --- | --- |
| 0,06 |  |

 | 10,32 |
| p- Terpineol |

|  |  |
| --- | --- |
| 3,51 |  |

 |

|  |  |
| --- | --- |
| 0,11 |  |

 | 1,15 |
| Total |

|  |  |
| --- | --- |
| 99,87% |  |

 |  98,84% | 99,20% |

Chemical analysis has shown the following values 99,87%, 98,84% and 99,20% components for the essential oil of Marrakech, Kenitra, and Taize respectively. The major component of the essential oil in Marrakech D-Limonene which has a rate of 39,49%. While the major component of the essential oil in Kenitra D-Limonene which has a rate of 29,19%.While the major component of the essential oil in Taize Carvacrol which has a rate of 10,32%.

**Determination of polyphenols content**

The standard curve (y = 0.028 x+0,0632 , r2 = 0.9994) for the determination of total polyphenol content was obtained by referring to a calibration curve carried out with gallic acid µg / ml (Table 3) ,(Figure 1),. The concentrations of total polyphenols obtained were presented in (Table 4) (Figure 2),they are expressed in µg EAG / g ES.

Table3: ABCORBANCE OF STANDARD ACIDGALIC

 Fig 1CALIBRATION CURVE OF STANDARD ACID GALIC

|  |  |
| --- | --- |
| **Conc. (μg/ml)** | **Absorbance at 765nm** |
| **500** | **1,45** |
| 250 | 0,761 |
| 125 | 0,431 |
| 62,5 | 0,251 |
| 31,25 | 0,145 |
| 15,625 | 0,094 |
|  7,81 | 0,075 |

**Table 4: The determination Polyphenol content of extracts ethanol and methanol of fresh Citrus limon peels**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample/****Extracts** | **Sample solution****µg/ml** | **Weight of dry****Extract** **mg/ml** | **Absorbance****at 765nm** | **GAE****Conc****C****µg/ml** | **GAE****Conc****C****mg/mL** | **TPC as****GAE****µg/ml** |
| E M | 1000 | 0.001 | 0,238 | 62,42857 | 0,0624 | 62,428571 |
| EK | 1000 | 0.001 | 0,224 | 57,42857 | 0,0574 | 57,42857 |
| EY | 1000 | 0.001 | 0,219 | 55,64285 | 0,0556 | 55,64285 |
| MM | 1000 | 0.001 | 0,227 | 58,5 | 0,0585 | 58,5 |
| MK | 1000 | 0.001 | 0,221 | 56,35714 | 0,0563 | 56,35714 |
| MY | 1000 | 0.001 | 0,213 | 53,5 | 0,0535 | 53,5 |

**Key** :*HE:essential oil ,EE: ethanol extract, EM :methanol extract*

**Fig 2: Determination Polyphenols content of extracts ethanol and methanol of fresh Citrus Limon peels**

Key:***EK****: ethanol kenitra****, EM*** *:ethanol marrkech,****EY:****ethanol yemen,*

*MM:***m***ethanol oil,****MK****: methanol kenitra****, MM*** *:methanol marrkech,****MY:m****ethanol yemen*

The content of total polyphenols in the ethanolic extracts shows different results,whose dominant is Marrakech by 62,42µg/ml, followed by the value of Kenitra 57,428 µg/ml and the lowest result in Yemen 55,642 µg/ml. And also for the content of total polyphenols in the Methanolic extracts, we find the high percentage in Marrakech by 58,562,42µg/ml, followed by the value of Kenitra 56,35714 µg/ml and the lowest result in Yemen 53,5 µg/ml. According to the results obtained in our study, the content of total polyphenols in the ethanolic extracts is the highest from the methanolic extracts. The reason the difference in polarity made the polyphenol ratio of extracted ethanol higher than that of extracted methanol, This study is conformity with (Jahanban-Esfahlan et al., 2019)16. As well, the differences in the values of total polyphenols content (TPC) for various citrus peel areas be affected by the degree of fruit ripening and genetic factors, environmental conditions, climate, lack of water, distance and proximity to the sea and elevation. This study is consistent with (Jahanban-Esfahlan et al.,, 2019)16.

**Determination of flavonoid**

The standard curve (y = 0.0043x+0,0282, r2 = 0.9705) for the determination of total flavonoid content was obtained by referring to a calibration curve carried out with Quercetin µg / ml (Table 5) (Figure 3).The concentrations of total flavonoid obtained were presented in (Table 6) (Figure 4)., they are expressed in µg EAG / g ES.

**Fig 3 CALIBRATION CURVE OFSTANDARD Quercetin glycoside**

 **Table5: Absorbance OF STANDARD Quercetin**

|  |  |
| --- | --- |
| **Conc. (μg/ml)** | **Absorbance at 420 nm** |
| 10 | 0,055 |
| 30 | 0,141 |
| 50 | 0,295 |
| 70 | 0,331 |
| 80 | 0,361 |
| 90 | 0,401 |
| 100 | 0,449 |

**Table 6: Determination flavonoid content of extracts ethanol and methanol of fresh Citrus limon peels**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample/****Extracts** | **Sample solution****µg/ml** | **Weight of dry****Extract** **mg/ml** | **Absorbance****at 765nm** | **GAE****Conc****C****µg/mL** | **GAE****Conc****C****mg/mL** | **TPC as****GAE****µg/ml** |
| E M | 1000 | 0.001 | 0,393 | 84,8372 | 0,0848 | 84,8372 |
| EK | 1000 | 0.001 | 0,269 | 56 | 0,056 | 56 |
| EY | 1000 | 0.001 | 0,219 | 44,37209 | 0,0443 | 44,37209 |
| MM | 1000 | 0.001 | 0,323 | 68,5581 | 0,0685 | 68,5581 |
| MK | 1000 | 0.001 | 0,224 | 45,53488 | 0,0455 | 45,53488 |
| MY | 1000 | 0.001 | 0,119 | 21,11627 | 0,0211 | 21,11627 |

**Fig 4:Calibrattion curve flavonoid content of extracts ethanol and methanol of fresh Citrus limon peels**

Key:***EK****: ethanol kenitra****, EM*** *:ethanol marrkech,****EY:****ethanol yemen,*

*MM:***m***ethanol oil,****MK****: methanol kenitra****, MM*** *:methanol marrkech,****MY:m****ethanol yemen*

The content of total flavonoid in the ethanolic extracts shows different results, who highest is Marrakech by84,8372 µg/ml, followed by the value of Kenitra 56 µg/ml and the lowest result in Yemen 44,37209 µg/ml. And also for the content of total polyphenols in the Methanolic extracts, we find the high percentage in Marrakech by 68,5581µg/ml, followed by the value of Kenitra 45,53488 µg/ml and the lowest result in Yemen 21,11627 µg/ml. According to the results obtained in our study, the content of total flavonoid in the ethanolic extracts is the highest from the methanolic extracts. The reason the difference in polarity made the flavonoids ratio of extracted ethanol higher than that of extracted methanol, This study is conformity with (Ngo, 20). As well The differences in the values of total flavonoid content (TFC) for various citrus peel areas be affected by the degree of fruit ripening and genetic factors, environmental conditions, climate, lack of water, distance and proximity to the sea and elevation. This study is consistent with (Jahanban-Esfahlan et al.,, 2019)16.

**Radical scavenging activities (DPPH)**

The Radical scavenging activities (DPPH) was found of standard ascorbic acid at different concentrations was found (Tables 7) (fig. 5). And also, The Radical scavenging activities (DPPH) of essential oil and extracts (Tables 8) (Fig 6,7,8). And that is by measuring the absorbance at the wavelength of 517 nm for different concentrations of extracts and the control.

**Table (7): % DPPH SCAVENGING ACTIVITY OF STANDARD ASCORBIC ACID**

**Fig (5): CALIBRATION CURVE OF STANDARD ASCORBIC ACID**

|  |  |
| --- | --- |
| Standard | Acid ascorbic |
| Con µg/ml |  Abcorbance517nm | SCV%  |
| 20 |  0,037 | 49,31 |
| 40 |  0,034 | 53,42 |
| 60 |  0,032 | 56,16 |
| 80 | 0, 031 | 57,53 |
| 120 |  0,029 | 60,27 |
| 140 |  0,028 | 61,64 |
| 160 |  0,026 | 64,38 |
| 180 |  0,022 | 68,49 |
| 200 | 0,022 | 69,86 |
| blank | 0,073 |  |

The DPPH radical scavenging activity of standard ascorbic acid was found to be highest at 200μl concentration which was 69.68%.

Table (8): The Radical scavenging activities (DPPH) of essential oil and extracts of freshCitrus limon peel in Marrakech, Kenitra, and Taize.

|  |  |  |  |
| --- | --- | --- | --- |
| **Extract sample** | **Marrakech** | **Kenitera** | **Taize** |
| **Eethanol** | **Eehtanol** | **Eehtanol** |
| Con µg/ml |  Abc517nm | SV% | IC50 µg/ml |  Abc517nm | SV% | IC50 µg/ml | Abc517nm |  SV%  | IC50 µg/ml |
| 20 |  0,282 | 6,622 | **92,04**  |  0,280 | 7,28 | **153,55** | 0,292 | 3,311 | **215,407** |
| 40 | 0, 248 | 17,88 | 0, 264 | 12,58 | 0,290 | 3,97 |
| 60 | 0, 223 | 26,15 | 0, 248 | 17,88 | 0,268 | 11,25 |
| 80 | 0,180 | 40,39 | 0,204 | 32,45 | 0,245 | 18,87 |
| 120 |  0,150 | 50,33 |  0,179 | 40,72 | 0,237 | 21,52 |
| 140 |   0,116 | 61,58 |   0,166 | 45,03 | 0,229 | 24,17 |
| 160 | 0, 108 | 64,23 | 0, 150 | 50,33 | 0,216 | 28,47 |
| 180 | 0, 096 | 68,21 | 0, 137 | 54,63 | 0,208 | 31,12 |
| 200 | 0, 069 | 77,15 | 0, 097 | 67,88 | 0,188 | 37,74 |
|  | 0, 302 |  | 0, 302 |  | 0,302 |  |
| **Extract sample** | **Marrakech** |  | **Kenitera** |  | **Taize** |  |
| **Methanol** |  | **Mehtanol** |  | **Mehtanol** |  |
| Conµg/ml |  Abc517nm | SV%  | IC50 µg/ml |  Abc 517nm | SV%  | IC50 µg/ml |  Abc | SV% | IC50 µg/ml |
| 20 |  0,301 | 16,38 | **145,6** |  0,335 | 6,94 | **179,17** |  0,345 | 4,16 | **274,899** |
| 40 |  0,238 | 33,72 |  0,325 | 9,72 |  0,331 | 8,05 |
| 60 |  0,210 | 40,27 |  0,289 | 19,72 |  0,323 | 10,27 |
| 80 |  0,190 | 47,22 |  0,285 | 20,83 |  0,305 | 15,27 |
| 120 |  0,137 | 61,94 |  0,241 | 33,05 |  0,254 | 29,44 |
| 140 |  0,105 | 70,83 |  0,212 | 41,11 |  0,244 | 32,22 |
| 160 |  0,086 | 76,11 |  0,195 | 45,83 |  0,222 | 38,33 |
| 180 |  0,064 | 82,22 |  0,176 | 51,11 |  0,221 | 38,61 |
| 200 |  0,062 | 82,77 |  0,168 | 53,33 |  0,189 | 47,5 |
|  |  0,360 |  |  0,360 |  |  0,360 |  |
| **Extract sample** | **Marrakech** |  | **Kenitera** |  | **Taize** |  |
| **HE** |  | **HE** |  | **HE** |  |
| Conµg/ml |  Abm517nm | SV%  | IC50 µg/ml |   Abm517nm | SV%  | IC50 µg/ml |  Abc517nm | SV% | IC50 µg/ml |
| 20 | 0,293  | 9,28 | **155,54** | 0,270 | 16,41 | **271,158** | 0,254 | 20,36 | **101,31** |
| 40 |  0,254 | 21,36 |  0,251 | 22,29 |  0,246 | 24,06 |
| 60 |  0,254 | 21,36 |  0,247 | 23,52 |  0,215 | 33,43 |
| 80 |  0,240 | 25,69 |  0,244 | 24,45 |  0,184 | 43,03 |
| 120 | 0, 232 | 28,17 | 0, 238 | 26,31 | 0, 132 | 59,13 |
| 140 |  0,177 | 45,2 |  0,229 | 29,11 |  0,107 | 66,87 |
| 160 |  0,157 | 51,39 |  0,202 | 37,46 |  0,083 | 74,31 |
| 180 |  0,117 | 63,77 |  0,198 | 38,69 |  0,066 | 79,56 |
| 200 |  0,117 | 63,77 |  0,186 | 42,41 |  0,061 | 80,11 |
|  | 0,323  |  | 0,323  |  | 0,323  |  |

**Fig (6): CALIBRATION CURVE OF DPPH SCAVENGING ACTIVITY OF EHTANOLIC EXTRACT**

**Fig (7): CALIBRATION CURVE OF SCAVENGING ACTIVITY OF MEHTANOLIC EXTRACT**

**Fig (9): CALIBRATION CURVE OF SCAVENGING ACTIVITY OF ESSENTAIL OIL**

The DPPH radical scavenging activity of ethanolic extract of fresh Citrus limon peel was found to be highest at 200μl concentration which was 77,15 %; 67, 88 %; 37,74%. Through it 50% inhibition (IC50) was obtained to be 92,04; 153,55 and 215,407 μg/ml in Marrakech, Kenitra, and Taize respectively. As well, the values radical scavenging activity of methanolic extract of the same concentration which are 82,77 %; 53,33 % and 47,5%. Through it 50% inhibition (IC50) was obtained to be 145,6; 179,17 and 274,899 μg/ml in Marrakech, Kenitra, and Taize respectively. but regarding, the values radical scavenging activity of essential oil of the same concentration which are 63,77 %; 42,19 % and 80,25%. Through it 50% inhibition (IC50) was obtained to be 155,54; 271,158 and 101,31 μg/ml in Marrakech, Kenitra, and Taize respectively.We find that the Marrakech region of extracted ethanolic is the highest value of polyphenols and flavonoids by 62,42 and 84,8372 µg/ml respectively, the lowest in the IC50 of value was 92,04µg/ ml. followed by Kenitra with value are 57,428 and 56 µg/ml, , and the value of IC50 is 153,55µg/ ml, the lowest value in Taize is 55,642 and 44,37209 µg/ml , and the value of IC50 is 215,407µg/ ml. According to the results obtained in our study, The antioxidant activity increases with increased polyphenols and IC50 deficiency in Marrakech, Kenitra, and Yemen. This study is consistent with Truong, ( Truong, Dieu-Hien - 2019)17. As well, the different values were due to genetic factors, fruit ripening, environmental conditions, climate, lack of water, distance,

proximity toThe sea and elevation. Also, This study is consistent with (Ghasemi, Kamran et al., 2019)18. Also, the difference in polarity made the yield ratio of extracted ethanol higher than that of extracted and methanol, so we find that citrus peel is more soluble in ethanol than other solvents. This is in conformity with the (Truong, Dieu-Hien - 2019)17

**Conclusions:**

The present study demonstrated the antioxidant activity increases with increased polyphenols and IC50 deficiency of fresh citrus limon peel in Marrakech, Kenitra, and Taize.

 The different values were due to genetic factors, fruit ripening, harvest season, environmental conditions, climate, lack of water, distance, proximity to the sea and elevation, as well the difference extracts in polarity.

**Reference**

1.R. Kummer, F. C. Fachini-Queiroz, C. F. Estevão-Silva, R. Grespan, E. L. Silva, C. A.Bersani-Amado, and R. K. N. Cuman, “Evaluation of Anti-Inflammatory Activity of Citrus MANUSCRIPT ACCEPTED ,**2015**.

2.Fao Faostat. Food and agriculture data**,**15 January **2019,**http:// www.fao.org/faostat/en/. Accessed on line**.**

3. S. S. Voo, H. D. Grimes, and B. M. Lange, “Assessing the biosynthetic capabilities of secretory glands in Citrus peel,” Plant Physiology**,2012,**vol. 159, pp. 81–94.

 4-A. F. Filipsson, J. Bard, and S. Karlsson, "Limonene", World Health Organization, **1998, 5th** edition, pp. 32.

5. R. Hirota, N. N. Roger, H. Nakamura, H. S. Song, M. Sawamura, and N. Suganuma, Anti-inflammatory Effects of Limonene from Yuzu (Citrus junos Tanaka) Essential Oil on Eosinophils,” Journal of Food Science**, 2010,**vol. 75, pp. H87–H92.

6.Del Rıo, J.A., Fuster, M.D., Gómez, P., Porras, I., Garc ́ ıa-Lidón, A., Ortuño, A. Citruslimon: a source of flavonoids of pharmaceutical interest. Food Chem. ,**2004,**84 (3), 457–461. https://doi.org/10.1016/S0308-8146(03)00272-3

7.Gil-Izquierdo, A., Riquelme, M.T., Porras, I., Ferreres, F.. Effect of the rootstock and interstock grafted in lemon tree (Citrus limon (L.) Burm.)on the flavonoid content of lemon juice. J. Agric. Food Chem. , **2004**,52 (2), 324–331. https://doi.org/10.1021/ jf0304775

8. Koh K.J., Pearce A.L., Marshman G., Finlay-Jones J.J., Hart P.H. Tea tree oil reduces histamine-induced skin inflammation. Br. J. Dermatol,**2002,**147:1212–1217. doi: 10.1046/j.1365-2133.2002.05034.x. [PubMed] [CrossRef] [Google Scholar]

9.Zou, Z., Xi, W., Hu, Y., Nie, C., & Zhou, Z.Antioxidant activity of Citrus fruits. Food Chemistry, **(2016),** 196, 885–896. doi:10.1016/j.foodchem.2015.09.072 url to share this paper:

sci-hub.tw/10.1016/j.foodchem.2015.09.072

## 10. Owosini A, Ayansina A, Amjo O,. In vitro assessment of the antimicrobial activities of leaf and stem extracts of Alchorneacordifolia. J ApplSci Environ Manag,2015,19(2):303. doi:10.4314/jasem.v19i2.18.

11. Ewansiha JU, Garba SA, Galadima M, Daniyan SY, Busari MB ,2016. Therapeutic Potency of Citrus Limon (L) Burm. F. (Lemon) Peel Extract Against Some Disease Causing Microorganisms. Int J Res Stud Biosci,**2020,** 4(11):30-39.https://www.arcjournals.org/ijrsb/volume-4-issue-11/6. Accessed January 19.

12.Adams R.Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy.Carol Stream. **,2005,**16:65-120.1.

13-Lister, E., & Wilson, P.Measurement of total phenolicsandABTS assay for antioxidant activity (personal communication).Crop Research Institute, Lincoln, New Zealand **(2001).**

14.Ordon, J.D., Gomez, M.A., Vattuone, M.I. (2006).Antioxi**Ordon, J.D., Gomez, M.A., Vattuone, M.I.Antioxidant activities of Sechiumedule (Jacq.) Swartz extracts. Food Chemistry, (2006), 97: 452–458.**dant activities of Sechiumedule (Jacq.) Swartz extracts. Food Chemistry, 97: 452–458.

15.Huang, H.B. Ke, J.S. He, X.Q. Ban, H. Zeng, Y.W. Wang Extracts of Haleniaelliptica exhibit antioxidant properties in vitro and in vivo Food Chem. Toxicol., 49 ,**(2011),** pp. 185-190

16.Jahanban-Esfahlan, Ali, AlirezaOstadrahimi, MahnazTabibiazar, and RyszardAmarowicz. “A Comparative Review on the Extraction, Antioxidant Content and Antioxidant Potential of Different Parts of Walnut (JuglansRegia L.) Fruit and Tree.” Molecules **,2019**, 24(11).

 17.Ghasemi, Kamran, Yosef Ghasemi, and Mohammad Ali Ebrahimzadeh. “ANTIOXIDANT ACTIVITY, PHENOL AND FLAVONOID CONTENTS OF 13 CITRUS SPECIES PEELS AND TISSUES.”, **2009**,Pak*. J. Pharm. Sci.* 5.

18.Truong, Dieu-Hien, DinhHieu Nguyen, Nhat Thuy Anh Ta, Anh Vo Bui, Tuong Ha Do, and Hoang Chinh Nguyen. 2019. “Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of SeveriniaBuxifolia.” Journal of Food Quality.Retrieved February 15, **2020,** (https://www.hindawi.com/journals/jfq/2019/8178294/).