

Evaluation of Antioxidant Activity of fresh Lemon (Citrus lemon L.) peel in Marrakech, Kenitra cities of Morocco and Taiz of Yemen

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Abstract: The principal objective of this study was to appraise the variation of antioxidant activity, total phenols, and total flavonoids of fresh lemon peel from regions located Marrakech and Kenitra in Morocco and a region Taiz in Yemen. The test of polyphenol was performed from extracts that were measured using the Folin-Ciocalteu technique. Also, an analysis of Flavonoid was performed of was measured by using the aluminum chloride. The Free radical scavenging activity of lemon extracts was measured by 2,2'-Diphenyl-1-picrylhydrazyl hydrate (DPPH).

The DPPH radical scavenging activity of ethanolic extract of fresh lemon peel found to be highest at 200 μ l 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 μ g/ml in Marrakech, Kenitra, and Taiz, 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 μ g/ml in Marrakech, Kenitra, and Taiz, respectively. But regarding the values radical scavenging activity of essential oil of the same concentration, which is 63.77%, 42.19%, and 80.25%. Through it, 50% inhibition (IC₅₀) was obtained to be 155.54; 271.158 and 101.31 μ g/ml in Marrakech, Kenitra, and Taiz, respectively. According to the results obtained in our study, the antioxidant activity increases with increased polyphenols and IC₅₀ deficiency in Marrakech, Kenitra, and Taiz.

Keywords: Citrus Limon; antioxidant activity; polyphenols; flavonoids; radical scavenging activity.

1. Introduction

Citrus is a variety of fruits and belongs to [Rutaceae family], it contains 52 species and 17 pieces ¹. The best known is lemon, mandarin, and sour orange, which have nutritional value and health benefits ². Citrus fruits also play an active role in preventing chronic diseases, diabetes, blood pressure, and some types of cancer ³. It is also present in cosmetics such as soaps, perfumes, shampoos, and toiletries ⁴. Citrus is classified as one of the most productive crops in the world, with an annual production of 102 \times 106 tons. Lemon represents the third most crucial citrus cultivation after oranges and mandarin, with global production reaching 420,000 tons annually ⁵.

Morocco, Portugal and Spain are citrus producers in the Mediterranean region, with an average annual production of 10 million tons ⁶.

Fruits and vegetables are natural sources of phenolic compounds (10). Therefore, the citrus peel contains flavonoids, phenolic compounds, essential oils, vitamins, and minerals ⁷⁻⁸.

It is well known that phenolic and secondary metabolites with associated double bonds usually exhibit antioxidant properties ⁹.

2. Experimental

Citrus fruits were obtained at the beginning of the

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harvest season in November 2019 from Morocco (Marrakech and Kenitra) and Yemen (Taiz). The study was conducted on the collected fruits at the Regional Center for Agricultural Research in Kenitra, Marrakech.

2.1. Preparation of Essential Oil

The essential oil content was determined by fresh Lemon peels by steam distillation¹⁰. The mixture was preheated for 6 hours at 4°C. Therefore, the yield was obtained by the relation between the mass of lemon peels and that of the essential oil.

2.2. Preparation of extracts

The solvents Ethanol and Methanol were extracted from the lemon peels using (Soxhlet technique). It was heated for 5 hours at 4°C, according to Lin study¹¹.

2.3. The chemical composition of fresh Citruslimon peel

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

2.3.1. Determination of total polyphenol content

The polyphenol was assessed of C.limon peels by the method the Folin-Ciocalteu according to Lister and Wilson¹³. 0,5 mL were accounted from the ethanolic extracts and methanolic extracts in Morocco exactly from Marrakech, Kenitra cities and from Taize city in Yemen, we added 4 mL of sodium carbonate (7.5 %, w/v) and added 2,5 mL of Folin Cio calteu 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).

2.3.2. Determination of Total Flavonoid content

The test flavonoids were performed of ethanolic and methanolic extracts of the fresh Lemon peels in Marrakech, and Kenitra Morocco cities and Yemen

Taiz city by the employment of the aluminum chloride colorimetric according to Ordon 14.0,5 mL of each sample was mixed with 0.5 mL of 0,2 aluminum chloride 10% and 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 of 40°C for 30 min. The absorbance measurements were synthetic, at 420 nm by utilizing 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).

2.4. Antioxidant Activity (AA)

The Free radical scavenging activity of citrus lemon extracts was measured by 2,2'-Diphenyl-1-picrylhydrazyl hydrate (DPPH)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 the 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.

3. Results

The results of our current study are illustrated clearly below the yield, composition chemical, polyphenols, and evaluation of Antioxidant Activity of fresh Lemon (Citrus lemon) peel in Marrakech and Kenitra cities Morocco and Yemen.

3.1. The yield of fresh lemon peel extracts

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 Marrakech, Kenitra, and Taize.

Extract	Marrakech			Kenitra			Taize		
	HE	EM	EE	HE	EM	EE	HE	EM	EE
Yield R %	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

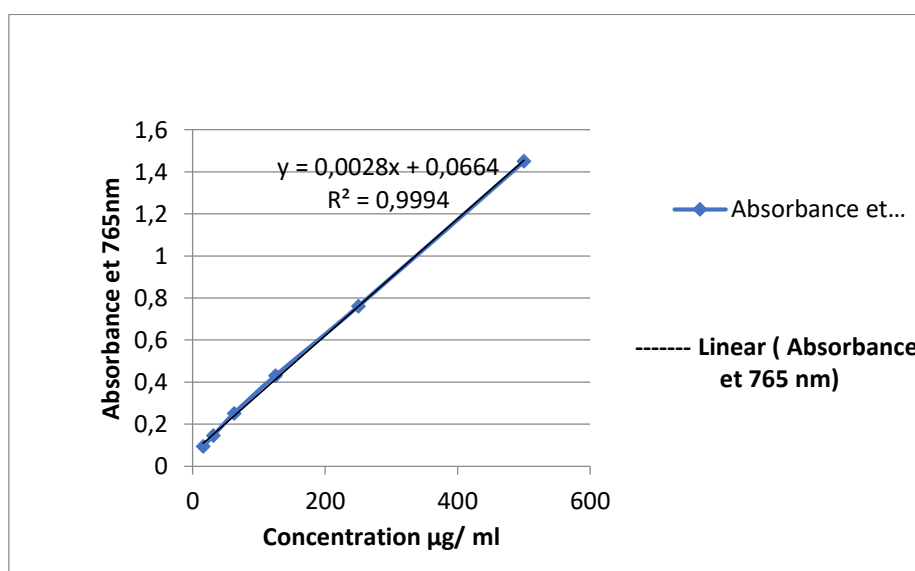
Table 2. Chemical compounds of essential oil of fresh Citrus limon peel for three cities Kenitra, Marrakech, and Taize.

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%.

3.2. Determination of polyphenols content

The standard curve ($y = 0.028x + 0.0632$, $r^2 = 0.9994$) for the determination of total polyphenol content was obtained by referring to a calibration curve carried out with gallic acid $\mu\text{g} / \text{ml}$ (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.

**Figure 1.** Calibration curve of standard Galic Acid**Table 3.** Absorbance of standard Acid Galic.

Conc. ($\mu\text{g}/\text{ml}$)	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 $\mu\text{g/ml}$	Weight of dry Extract	Absorbance at 765nm	GAE ConcC $\mu\text{g/ml}$	GAE ConcC mg/mL	TPC as GAE $\mu\text{g/ml}$
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

EM: Ethanol extract Marrakech MM: Methanol extract Marrakech

EK: Ethanol extract Kenitra MK: Methanol extract Kenitra

MY: Methanol extract Yemen EY: Ethanol extract Yemen

The content of total polyphenols in the ethanolic extracts shows different results, whose dominant is Marrakech by $62.42\mu\text{g/ml}$, followed by the value of Kenitra $57.428\mu\text{g/ml}$ and the lowest result in Yemen $55.642\mu\text{g/ml}$. Also, for the content of total polyphenols in the Methanolic extracts, we find a high percentage in Marrakech by $58.562\mu\text{g/ml}$, followed by the value of Kenitra $56.357\mu\text{g/ml}$ and the lowest result in Yemen $53.5\mu\text{g/ml}$.

3.3. Determination of flavonoid

The standard curve ($y = 0.0043x + 0.0282$, $r^2 = 0.9705$) for the determination of total flavonoid content was obtained by referring to a calibration curve carried out with Quercetin $\mu\text{g} / \text{ml}$ (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.

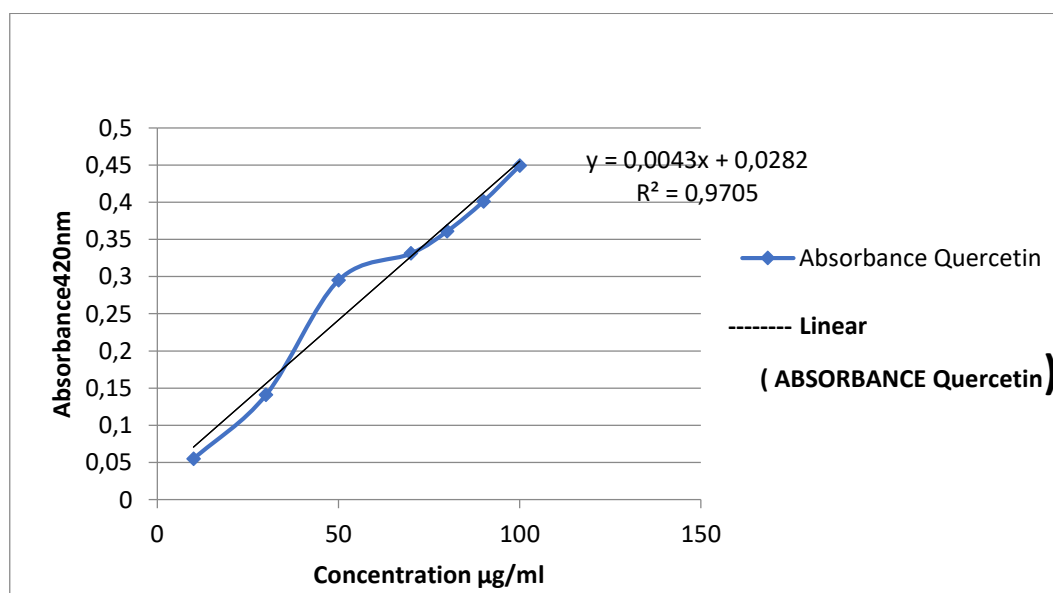
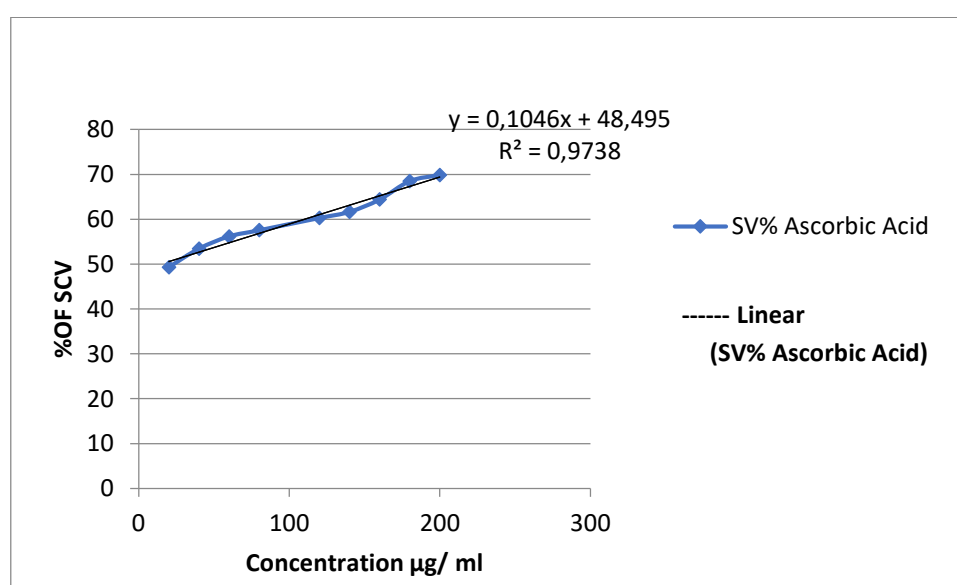
**Figure 2.** Calibration curve of standard Quercetin glycoside

Table 5. Absorbance of standard Quercetin.

Conc. ($\mu\text{g/ml}$)	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 $\mu\text{g/ml}$	Weight of dry Extract mg/ml	Absorbance at 765nm	GAE ConcC $\mu\text{g/mL}$	GAE ConcC mg/mL	TPC as GAE $\mu\text{g/ml}$
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

**Figure 3.** Calibration curve of standard Ascorbic Acid

The content of total flavonoid in the ethanolic extracts shows different results in [Table 6](#) and [Figure 4](#); the highest one is Marrakech by 84.837 $\mu\text{g/ml}$, followed by the value of Kenitra 56 $\mu\text{g/ml}$ and the lowest result in Yemen 44.372 $\mu\text{g/ml}$. Also, for the content of total polyphenols in the Methanolic extracts, we find a high percentage in Marrakech by 68.558 $\mu\text{g/ml}$, followed by the value of Kenitra 45.534 $\mu\text{g/ml}$ and the lowest result in Yemen 21.116 $\mu\text{g/ml}$.

3.4. Radical scavenging activities (DPPH)

The Radical scavenging activities (DPPH) were found of standard ascorbic acid at different concentrations was found ([Tables 7](#)) ([Figure 5](#)). Also, The Radical scavenging activities (DPPH) of essential oil and extracts ([Table 8](#)) ([Figure 6, 7](#)). That is, by measuring the absorbance at the wavelength of 517 nm for different concentrations of extracts and the control.

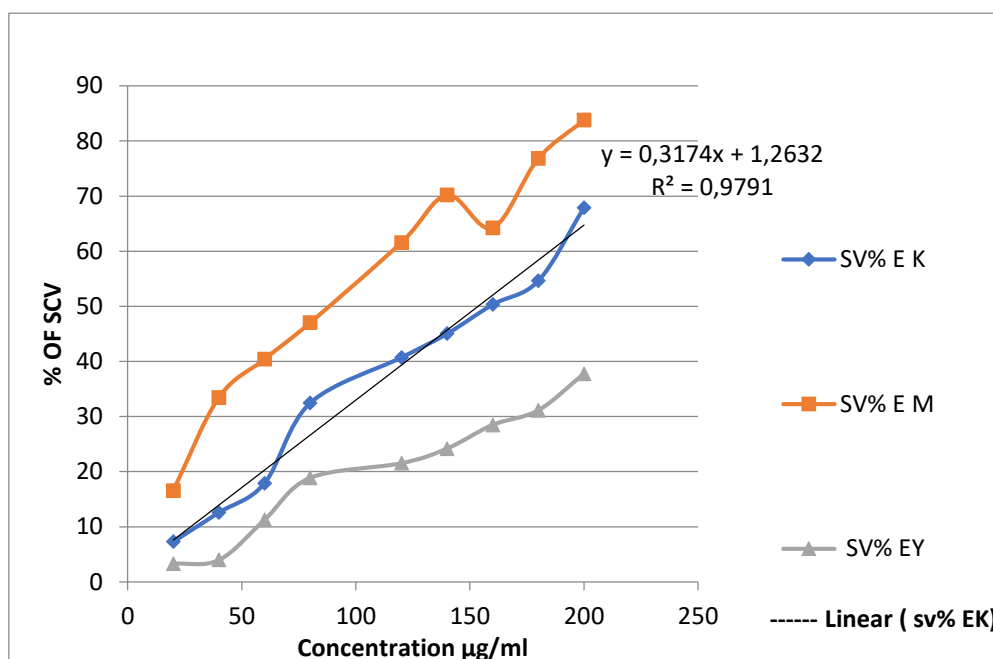


Figure 4. Calibration curve of DPPH scavenging activity of ethanolic extract of fresh citrus limon peels for three regions.

Table 7. % DPPH Scavenging Activity of standard Ascorbic Acid.

Standard Con µg/ml	Acid ascorbic	
	Absorbance 517nm	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 fresh Citrus limon peel in Marrakech, Kenitra, and Taize (Yemen).

Extract sample Con µg/ml	Marrakech			Kenitera			Taize		
	Ethanol			Ehtanol			Ehtanol		
	Abc 517nm	SV%	IC50 µg/ml	Abc 517nm	SV%	IC50 µg/ml	Abc 517nm	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		
Con µg/ml	Abc 517nm	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		
Con µg/ml	Abm 517nm	SV%	IC50 µg/ml	Abm 517nm	SV%	IC50 µg/ml	Abc 517nm	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		

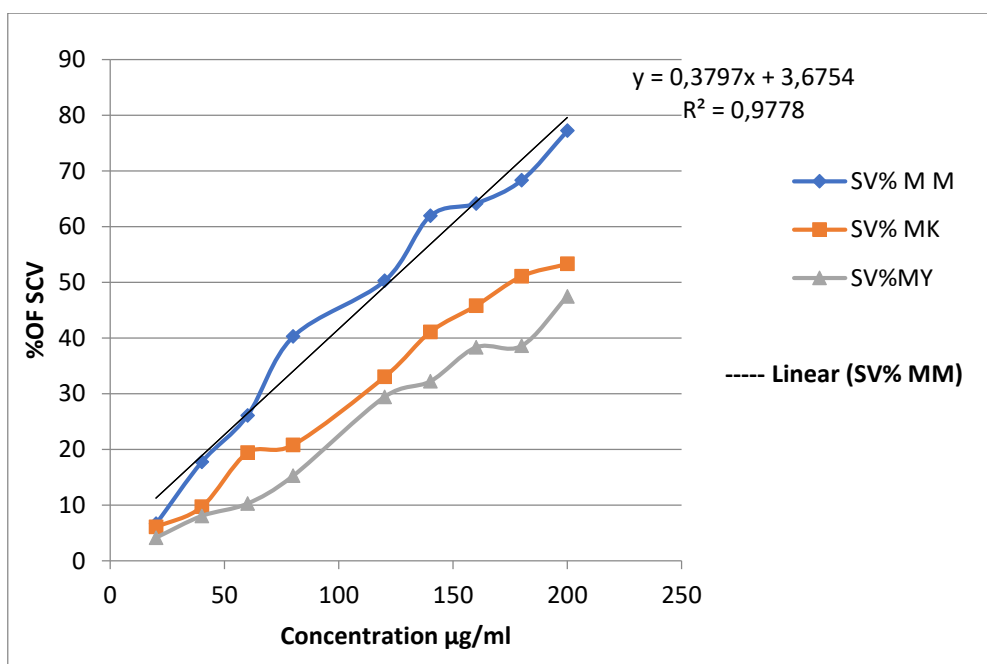


Figure 5. Calibration curve of scavenging activity of methanolic extract of fresh citrus limon peels for three regions.

SV%EM: percentage scavenging activity of Ethanol extract Marrakech

SV%EK: percentage scavenging activity of Ethanol extract Kenitra

SV%EY: percentage scavenging activity of Ethanol extract Yemen

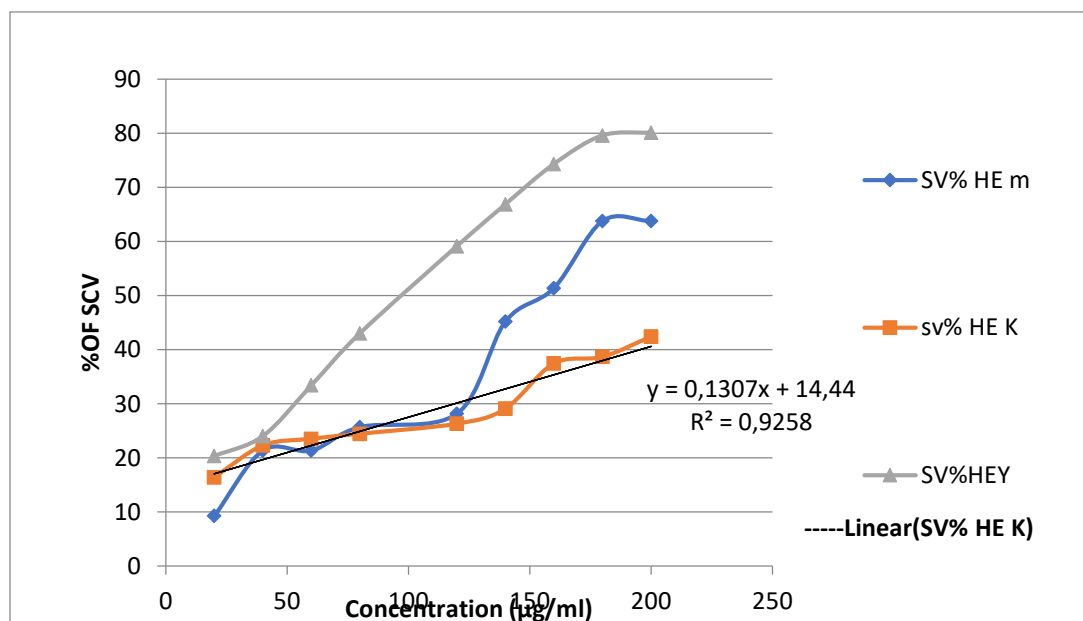


Figure 6. Calibration curve of scavenging activity of essential oil of fresh citrus limon peels for three regions.

SV%MM: percentage scavenging activity of Methanol extract Marrakech

SV%MK: percentage scavenging activity of Methanol extract Kenitra

SV%MY: percentage scavenging activity of Methanol extract Yemen

4. Discussion

It is clear from the obtained results in Table 1. The yield rate of essential oil was (0.90%) in Yemen higher than the yield in the Marrakech, it was (0.78%) higher than the yield in the Kenitra .it was (0.64 %).

While the yield rate of extracting methanolic (EM) and extracted ethanolic (EE), it was (9.8%) and (10.05%) in Marrakech higher than the yield in the Kenitra, it was (8.3%) and (8.9%) in Kenitra higher than the yield in the Taiz, it was (8.2%) and (8.6%) respectively. Therefore, the yield ratio of extraction

ethanol is higher than extraction methanol and essential oils.

The DPPH radical scavenging activity of ethanolic extract of fresh Citrus limon peels 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 Taiz, 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 (Marrakech obtained to be (92.04); (153.55) and (215.407) µg/ml in Marrakech, Kenitra, and Taiz, 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 Taiz 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 Taiz respectively (Table 8 and Figures 4,5,6). We found that the Marrakech region of extracting ethanolic is the highest value of polyphenols and flavonoids by (62.42) and (84.837) µg/ml, respectively, the lowest in the IC50 of value was (92.04) µg/ml. Followed by Kenitra with value are (57.428) and (56) µg/ml and the value of IC50 is (153.55)µg/ml, the lowest value in Taiz is (55.642) and (44.372) µg/ml and the value of IC50 is (215.407)µg/ml. Through the results which reached, we find that the oxidation activity and the polyphenols and flavonoids of citrus peels in the Marrakech and Kuneitra region in Morocco are higher than the Taiz region in Yemen.

The reason is due to the influence of citrus fruits of lack of water, environmental conditions, genetic factors, genetic makeup, climate, and distance from the sea.

As well, the content of total polyphenols in the ethanolic extracts is the highest than from the methanolic extracts. This study is conformity with Hegazy Ibrahim (2012) ¹⁶. Who determined a higher value of phenolic (mg/g of extract) in Ethanol (169.38) extract rather than Methanol (165.38) extract of orange peel.

The reason is the difference in polarity that made the polyphenol ratio of extracted Ethanol higher than extracted Methanol. Therefore the type of extraction solvent and its polarity may have a significant impact on the level of extracted polyphenols. The polarities of the polyphenols range from polar to non-polar; optimum extraction of polyphenols is usually obtained in the polar solvent, which has a better efficiency of solvation as a result of interactions

(hydrogen bonds) between the polar sites of the antioxidant compounds and the solvent than non-polar one ¹⁷. This study differed with Truong, who determined a higher value of phenolic (mg/g of extract) in Methanol (13.36) of other solvents extract rather than methanol (165.38) extract of *Severinia buxifolia*. The reason is that plant materials have a difference in the solubility of polar compounds ¹⁸.

5. Conclusion

The present study demonstrated the antioxidant increases activity with increased polyphenols and IC50 deficiency of fresh citrus lemon peel in Marrakech, Kenitra, and Taiz, as well as the difference in the regions which affect the extraction of polyphenols and flavonoids, as well as the oxidation activity. This is the result of environmental conditions, climate, lack of water, and height from the sea, genetic factors, and genetic makeup. In addition to the type of polar solvent, the choice of plant's appropriate solvent and also the harvest season, all affect the result of extraction.

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