



Evaluation of Antifungal Activity of Lemon (Citrus lemon) in Marrakech and Kenitra cities Morocco



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Abstract

The main objective of this study is to evaluate antifungal activity by using fresh lemon peels in two cities: Marrakech and Kenitra, Morocco. This fungal activity was tested against seven pathogenic fungal strains which are *Saccharomyces cerevisiae*, *Candida albicans*, *Candida spp1*, *Candida spp2*, *Candida spp3*, *Candida spp4*, and *Candida spp5*. The study used, in this procedure, the extract of ethanol and methanol, besides the extraction of essential oils of lemon peels in the Marrakech and Kenitra in order to obtain the yield, the minimum inhibitory concentrations (MIC), and the minimum concentrations of antifungals (MFC). As a result, the yield of essential oil, methanol, and ethanol extracts were 0.78%; 9.8%; 10.05%, and 0.64%, 8.3%, 8.9% in Marrakech, Kenitra, respectively. The minimum inhibitory concentrations (MIC) were tested at concentrations ranging from (0.1, 0.25, 0.5, 1.25 and 2.5 mg/ml) as well as their the minimum concentrations of antifungals (MFC). Also, Zones of inhibition were recorded extend from 9 to 36 mm and from 8 to 18 mm in the concentrations of ethanolic extracts, the zones inhibition ranged from 10 to 26 mm and from 9 to 18 mm in the concentrations of methanolic extracts, and the zones inhibition ranged from 20 to 34 mm and 10 to 20 in the concentrations of essential oil for Marrakech and Kenitra, successively. The minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) to the lower concentration, as opposed to *Saccharomyces cerevisiae*, is 0.1 mg/ml, minimum inhibitory concentrations (MIC) and minimum antifungal, concentrations (MFC) to higher concentration against *Candida spp1* are 2.5 mg/ml). The results showed a difference in yields due to the difference in solvents and also in regions. The highest antifungal potentiality was exhibited by the ethanol followed by the methanol followed by the essential oil. Therefore, increased yield offset by high fungal activity, because of the difference in environmental conditions, climate, lack of water, distance, proximity to the sea and elevation, genes, extraction and season. The activities of the extracted oils depend on the availability of the active constituent based on the use of a solvent.

Keywords: Lemon peel; Antifungal; Essential oil; The minimum Inhibitory Concentration (MIC); Minimum fungal concentration (MFC)

1. Introduction

Researches demonstrated a significant advance in discovering the antibiotic; it has been achieved worth results in treating bacterial diseases, microbial infections, antioxidants, the types of resistance and its mechanisms.

The bacteria which resists the antibiotic and the lack of understanding this resistance poses a substantial threat regarding the morbidity and the mortality on

the worldwide with the increased rate of using antibiotic, particularly for multi-drug resistant (MDR).

The Infections are increasing significantly of *Candida* infection; it greatly affects people who suffer for immune deficiency, children, elderly, individuals hospitalized in Intensive Care Units (ICU), and users of invasive devices [1]. Multidrug-resistant (MDR) organisms have become a global health concern

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affecting the diagnosis, treatment, and prevention [2]. Likewise, the emergence of Covid-19 is a viral disease of positive-sense single-stranded RNA viruses of the family Coronaviridae (subfamily Coronavirinae), is an animal product that is called the emerging coronavirus, it is similar to the SARS virus[3], which is considered an acute respiratory disease that appeared in 2019 in Wuhan, China, and spread around the world, causing a global pandemic and out of control, killing millions of people and killing millions, including all ages. At the beginning of the pandemic, vaccines and anti-virus drugs were not available to treat human and animal coronavirus infection [4-5]. This was a motivation to look for new alternatives in order to control the microbial infection using the essential oils in the plant. The comital oils consist of complex molecules that containing on 150 molecules on average including monoterpenes, sesquiterpenols, sesquiterpenes, esters, aldehydes, oxides, phenols, coumarone, ketones or others which appear only in the form of traces[6]. There are essential oils in all members of the plant but with varying amounts in leaves, fruits, bark, pox, wood, heart, gum, balsam, berries, thyme, flowers, twigs and buds. They contain natural organic compounds and metabolites. The quality, quantity, and nature of plant organs that have produced through hereditary pathways within the cells depend on the factors of agriculture, environment, genes, climate, soil conditions, the time of harvest and finally handling post-harvest before isolation[7].

. It was our current study of citrus peels (Citrus Lemon). From (Rutaceae) is one of the most widely used agricultural products in the world. The citrus composing about 40 species that are distributed around in the world [8]. produced annually estimated by 100 million tons of citrus fruits therefore making the citrus the largest family contributor to the world's fruit production[9].

Citrus peels contain a set of secondary ingredients ascorbic acid, polyphenols, flavonoids, and carotenoid compounds with substantial antioxidant activity[10, 11]. In addition to that, Its fruits contain vitamin C, folic acid, potassium, and pectin. Citrus species have contributed to resistance to life-threatening diseases[12]. Citrus lemon has already demonstrated antifungal activity against candida infection[13]. The results of our study showed that fresh citrus peel has antifungal activity against

laboratory candida such as *saccharomyces cervicae*, *candida albicans*, *candida spp1*, *candida spp2*, *candida spp3*, *candida spp4*, and *candida spp5*.

2. Materials and methods

Plant material: The fruits of citrus were harvested at the beginning of the harvest in November 2019 from Morocco (Marrakech, Kenitra). The collected material was identified in the regional centre 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.

2.1. The Extraction of Essential Oil (HE)

Samples of 200.22 g and 200.24 g of fresh lemon peels in Morocco (Marrakech, Kenitra) were extracted by hydro distillation using a Clevenger apparatus Clevenger [14] respectively. The mixture was preheated oven for 7 hours. The Yield Analysis was measured with the comparison between the mass of lemon peels and the mass of the essential oil.

2.2. Soxhlet extraction

Samples of 200.22 g and 200.24 g of fresh lemon peels in Morocco (Marrakech, Kenitra) were extracted by Soxhlet respectively. Extracted perfectly with 600 ml the each solvent individually by employing the extraction apparel for 5 h at a temperature not exceed the boiling point of the Solvent according to the study of Lin et al, (1999) [15]. Our solvents are Ethanol and Methanol, The filtration of extract was made and concentrated to dryness. Yield of the extract obtained was calculated as follows: $R (\%) = M_1 / M_2 \times 100$ Where: R: yield of essential oil M1: the mass of the essential oil obtained in g M2: the mass of Limon peels g.

2.3. Analysis of the chemical composition of lemon peel

Test of essential Oil was performed by employing gas chromatography-mass spectrometry (GC-MS) along with the mass spectrometer (Q-8 MS ion trap) by application of Adams data [16]. And the preliminary phytochemical analysis was a fresh lemon peel extracts, were subjected to preliminary

phytochemical screening following the methodology [17,18,19], that showed the existence of alkaloids, saponin, sterols, Steroids, terpenoids, protein and amino acid, tannins, carbohydrates.

2.4. Antifungal activity assay

Overnight cultures of the *Saccharomyces cerevisiae*, *Candida albicans*, *Candida spp1*, *Candida spp2*, *Candida spp3*, *Candida spp4*, and *Candida spp5* were prepared on potato dextrose agar media. They were obtained from the Laboratory of Institute of Health Sciences, Rabat City, Morocco. This work was done according to a template [14]. So we used new suitable broth medium to test laboratory living organisms, and that to obtain the standard turbidity of 0.5 McFarland's by applying sterile saline and the lap of cultures at a temperature of 37 °C. Then we filled and dried the surface of Mueller–Hinton agar plate, we used sterile corn borer to drill wells with a diameter of 5mm approximately 2cm, and in the drilling area we put extracts for fresh lemon peels. Then the plates were placed in the refrigerator for 72 hours and for an hour before incubation.

2.5. Measurement of Minimum inhibitory concentration (MIC)

The minimal concentration inhibition (MIC) inhibition technique was chosen with the lowest concentration of the extract that did not significantly grow to see inhibitory concentrations according to the Oceanian and age model (2010) [20].

This was done by examining the organisms that have a susceptibility to fresh lemon peel extracts in the broth, also preparing different concentrations for each extract 0.25, 0.5, 2.5 and 1.25 mg/ml). It was incubated at 37° C for 24 hours, for that we picked the minimal concentration that did not significantly grow to see inhibitory concentrations (MIC).

2.6. Measurement of Minimum fungicidal concentration (MFC)

When the plates incubated in 24 hours at 37°C, we observed that no development of the fungicidal, so we conclude the extracts concentration who shows growth as the minimum fungicidal concentration (MFC).

3. Results

3.1. 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 by steam distillation using a Clevenger. Also, we got the extracted methanol EM, and the extracted ethanol EE by using Soxhlet. They were obtained the yield rate of HE, EM, and EE was 0.78%; 9.8%; 10.05%, and 0.64%; 8.3%; and 8.9% in Marrakech, Kenitra respectively.

Table 1: The yield of fresh lemon peel

Extract	Marrakech			Kenitra		
	HE	EM	EE	HE	EM	EE
Yield R %	0.78	9.8	10.05	0.64	8.3	8.9

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

It is clear from the results obtained in Table 1. The yield rate of extraction HE, EM and EE was 0.78%; 9.8% and 10.05% in Marrakech higher than the yield in the Kenitra was 0.64%, 8.3%, and 8.9% respectively. Also, the yield ratio of extraction ethanol is higher than that of extraction methanol and essential oils.

3.2. Chemical analysis

It has shown the following values 99, 87%, 98, 84% components for the essential oil of Marrakech, Kenitra 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%. The important components of Table 2, fig 1, 2

3.3. Phytochemical analysis

Table (3) showed phytochemical Properties of fresh lemon peel Extract (ethanolic, methanolic) positive results for alkaloids, saponin, sterols, Steroids, terpenoids, protein, amino acid, tannins, and carbohydrates in Morocco. The same results showed chemical properties of lemon peels in India [21].

Table 2: Chemical compounds of essential oil of fresh lemon peel that is grown in Morocco (Marrakech, Kenitra).

Chemical compounds	Marrakech	Kenitra
	Citrus lemon HE	Citrus lemon HE
	P %	P %
<i>β-Myrcene</i>	1,20	2,67
<i>D-Limonene</i>	39,49	29,19
<i>Linalyl Acetate</i>	14,07	14,54
<i>α-Terpineol</i>	4,90	10,47
<i>α-Pinene</i>	7,93	0,64
<i>β-Pinene</i>	5,54	4,69
<i>Carvacrol</i>	1,25	0,06
<i>p- Terpineol</i>	3,51	0,11
Total	99,87%	98,84%

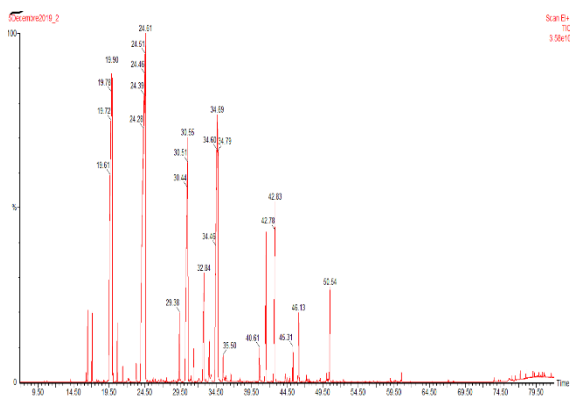


Fig.1 GC-MS analysis of essential oil of lemon peels in kenitra

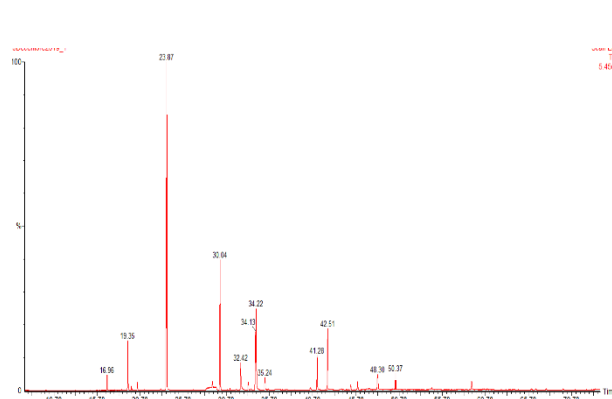


Fig.2 GC-MS analysis of essential oil of lemon peels in Marakech

Table3. Phytochemical Properties of fresh lemon peel Extracts in Marrakech, Kenitra

fresh lemon Extracts	Phenols	Flavonoid	Alkaloids	Tannins	Cardiac glycosides	Steroids	Terpenes	Volatile oil	Anthraquinon	Saponins
EE	+	+	+	+	+	+	+	+	+	+
ME	+	+	+	+	+	+	+	+	+	+

Key: HE: essential oil, EE: ethanol extract, EM :methanol extract, (+): Present

3.4. Antifungal activity assay

Our results yielded in (Table 4 and Table 5) shows that lemon peel has activity against laboratory fungal race. The minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) towards concentrates opposite of antifungal races regulated from (0.1, 0.25, 0.5, 1.25 and 2.5 mg/ml). Zones of inhibition were recorded of the citrus peel extracts against seven fungal races. When we compare between

Marrakech and Kenitra, we find: For Marrakech, the zones of inhibition extend from 9 to 36 mm in the concentrations of extracted ethanolic. Minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) for lower concentrate against *saccharomyces cerevisiae* are 0.1 mg/ml). It was higher inhibition zones 36 mm. As for, the *candida spp1* has observed inhibition zones 9 mm. It was less susceptible to having the inhibitory MIC, fungicidal MFC with a higher concentration 1.25 mg/ml. The zones of inhibition extend from 26 to 10 mm in the concentrations of

extracted methanolic. The minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) towards lower concentrate opposite of *saccharomyces cerevisiae* are 0.25, 0.5 mg/ml) respectively. It was higher inhibition zones 26 mm. As for, the *candida spp1* has observed inhibition zones 10 mm. It was less susceptible to having the inhibitory MIC, fungicidal MFC with a higher concentration are 2.5 mg/ml. the zones of inhibition extend from 20 to 34 mm in the concentrations of essential oil. The Minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) to lower concentrate against *saccharomyces cerevisiae* are 0.1 mg/ml. It was higher inhibition zones 34 mm. As for, the *candida spp2* has observed inhibition zones 20 mm. It was less susceptible to having the inhibitory MIC; fungicidal MFC with a higher concentration is 0.25 mg/ml.

We find: For Kenitra, inhibition zones extend from 8 to 18 mm in the concentrations of extracted ethanolic. Minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) to lower concentrate opposite of *saccharomyces cerevisiae* are 0.5 mg/ml). It was higher inhibition zones 18 mm. As for, the *candida spp1* has observed inhibition zones 8 mm. It was less susceptible to having the inhibitory MIC; fungicidal MFC with a higher concentration is 2.5 mg/ml. The zones of inhibition extend from 9 to 18mm in the concentrations of extracted methanolic. The Minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) to lower concentrate opposite of *Saccharomyces Cerevisiae* are 0.5 mg/ml) respectively. It was higher inhibition zones 18 mm. As for, the *candida spp4* has observed inhibition zones 9mm. It was less susceptible to having the inhibitory MIC; fungicidal MFC with a higher concentration is 2.5 mg/ml. The Zones inhibition extends from 10 to 20 mm in the concentrations of essential oil. Minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) for lower concentrate against *Saccharomyces Cerevisiae* are 0.25 mg/ml. It was higher inhibition zones 20 mm. As for, the *candida spp* has observed inhibition zones 10 mm. It was less susceptible to having the inhibitory MIC;

fungicidal MFC with a higher concentration is 2.5 mg/ml.

4. Discussion

It is clear from the results obtained in Table 1. The difference of environmental conditions, climate, lack of water, distance, proximity to the sea and elevation were identified between Marrakech and Kenitra. In that, you make the yield rate of extracts of citrus limon in Marrakech higher than the yield rate of extracts in Kenitra. This is in conformity with the report [22]. Also, the difference in polarity made the yield ratio of extracted ethanol higher than that of extracted methanol and essential oils, so we find that citrus peel is more soluble in ethanol than other solvents. This is in conformity with the report [23].

It is clear from the results obtained in Table 2. There is a difference in the values of chemical analysis in the major component of the essential oil and the important components. While the main ingredient is D-Limonene of lemon peel in Marrakech and Kenitra. The obtained results in this work are similar to the work that is reported by employers in Algeria [24]. The number of chemical compounds is 33, limonene (7.18%) was the main component. Also, this is in conformity with the report of eastern Morocco [19]. Illustrated that the number of chemical compounds is 15, D- Limonene 88.97% was the main component. This is in conformity with the report [25]. Illustrated that the number of chemical compounds 13, limonene (51.40%) was the main component, and also in Iran [26]. illustrated that the number of chemical compounds is 21 also limonene (61.4%) was the main component.

In short, citrus peels contain a group of important compounds. In order to preserve them, the appropriate harvest should be chosen, as it is responsible for their preservation and their percentage.

Phytochemical constituents of citrus lemon peel such as tannins, alkaloids, flavonoids, phenolic compounds, and several other aromatic compounds are secondary metabolites that were used in achieving a defines mechanism against the antifungal activity.

It is clear from the results obtained in Table (4) and Table (5) that citrus peel extracts showed

significant antifungal activity against all the tested organisms. Whereas, the test organisms interacted differently with peel extracts .The minimum inhibitory concentrations (MIC) and minimum antifungal concentrations (MFC) towards concentrates opposite of antifungal races regulated from (0.1, 0.25, 0.5, 1.25 and 2.5 mg/ml). Zones of inhibition were recorded of the citrus peel extracts against seven fungal races.

We find that the lemon peel extracts of Marrakech were the most active compared to the

lemon peel extracts of kenitra. The results of this study show that increased yield was matched by high fungal activity. The results of this study show also that the ethanolic extracts of the peel of the fruit of Citrus lemon were highly successful in producing the desired result against most of the fungal strains. Therefore, the activity of the extract depends on the availability of the active constituent based on the use of a solvent. This is in conformity with [15].

Table 4: Zones of Inhibition of fresh Lemon Peel extracts (mm) against some fungal strains in Marrakech ,Kenitra

Zone of inhibition (mm) of fresh lemon peel						
Fungal Tests	Marrakech			Kenitra		
	HE	EM	EE	HE	EM	EE
Saccharomyces Cerivicae	34	26	36	20	18	18
Candida Albicans	25	13	15	18	13	17
Candida SPP1	22	10	9	17	11	8
Candida SPP2	20	11	17	10	11	11
Candida SPP3	30	20	24	15	10	12
Candida SPP4	23	18	24	13	9	13
Candida SPP5	20	16	25	11	22	16

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

Table5: Antifungal activity of fresh lemon peel in Marrakech ,Kenitra

Antifungal activity of fresh lemon peel												
Fungal Tests	Marrakech						Kenitra					
	HE		EM		EE		HE		EM		EE	
	MIC mg/ml	MFC mg/ml	MIC mg/ml	MFC mg/ml	MIC mg/ml	MFC mg/ml	MIC mg/ml	MFC mg/ml	MIC mg/ml	MFC mg/ml	MIC mg/ml	MFC mg/ml
Saccharomyces Cerivicae	0.1	0.1	0.25	0.5	0.1	0.1	0.25	0.25	2.5	2.5	0.5	0.5
Candida Albicans	0.25	0.25	1.5	1.5	1.25	2.5	0.5	0.5	2.5	2.5	0.25	0.25
Candida SPP1	0.25	0.25	2.5	2.5	1.25	1.25	0.5	0.5	1.25	1.25	2.5	2.5
Candida SPP2	0.25	0.25	2.5	2.5	0.5	0.5	2.5	2.5	2.5	2.5	2.5	2.5
Candida SPP3	0.1	0.25	0.25	0.5	0.5	0.5	1.25	1.25	2.5	2.5	2.5	2.5
Candida SPP4	0.25	0.5	0.25	0.5	0.5	0.5	1.25	1.25	2.5	2.5	2.5	2.5
Candida SPP5	0.25	0.5	0.25	0.5	0.5	0.5	1.25	1.25	1.25	1.25	2.5	2.5

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

MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration

5. Conclusion

Citrus fruits are the largest contribution to the production of fruits in the world, as citrus fruits have contributed to many fields, including traditional treatment, in the treatment against bacterial and fungal infection, pharmaceuticals, and cosmetics. Based on these results, the present study demonstrated that citrus lemon peel has significant antifungal activity against *Saccharomyces cerevisiae*, *candida albicans*, *candida spp1*, *candida spp2*, *candida spp3*, *candida spp4*, and *candida spp5*. show that increased yield was matched by high fungal activity, because of the difference in environmental conditions, climate, lack of water, distance, proximity to the sea and elevation, genes, extraction and season. The activity of the extract depends on the availability of the active constituent based on the use of a solvent. From these results, more studies are needed in the future, how to use appropriate solvents in the extraction and how to preserve them and not damage them in order to benefit more from them in various fields and requirements of life.

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