

## ANTIBACTERIAL AND ANTIFUNGAL EFFICACY OF CITRUS PEEL ESSENTIAL OILS FROM FOUR MOROCCAN CITRUS VARIETIES

Loubna Ouahmane<sup>1</sup>, Mohammed Abdessadek<sup>1</sup>, Anass Kchikich<sup>1</sup>, Bouchra Bouftila<sup>1</sup>, Karim Sbihi<sup>1</sup>, Hasna Boura<sup>2</sup>, Oukacha Amri<sup>1,3</sup> and Belkassem El Amraoui<sup>1</sup>

<sup>1</sup>Laboratory of Analysis, Modeling, Engineering, Natural Substances and Environment, Polydisciplinary Faculty of Taroudant, Ibn-Zohr University, Taroudant, Morocco

<sup>2</sup>Laboratory of Helicobacter Pylori and Gastric Pathologies, Pasteur Institute of Morocco, Casablanca, Morocco

<sup>3</sup>Laboratory of Plant Biotechnologies (BioTechV), Faculty of Sciences, Ibn Zohr University of Agadir, Morocco

### ABSTRACT

**Background.** Infections brought on by bacteria and fungi pose a serious risk to public healthcare worldwide. The emergence of virulent and resistant microbes has weakened the effectiveness of several antimicrobial therapies. As a result, natural compounds are becoming more and more popular as substitute antibacterial agents. Citrus peels and other by-products are known to contain essential oils with potential biological benefits.

**Objective.** The current study examines the antimicrobial characteristics of four Moroccan citrus peel essential oils *in vitro*.

**Material and Methods.** Citrus peels were hydrodistilled to extract the essential oils. Antimicrobial properties were assessed by applying the agar disk-diffusion technique. Minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC) were ascertained by the broth microdilution method.

**Results.** The extraction yield of citrus peels ranged from 0.4% to 1.0%. All essential oils have demonstrated antimicrobial activity. *Citrus clementina* was the most effective against *Salmonella* Typhimurium sp. ( $33.33 \pm 2.08$  mm), while the essential oil of *Citrus sinensis* demonstrated the strongest antibacterial activity against *Bacillus* sp. ( $24.50 \pm 0.71$  mm), *Staphylococcus aureus* ( $21.00 \pm 2.65$  mm), *Enterococcus faecalis* ( $19.00 \pm 1.00$  mm), and *Pseudomonas fluorescens* ( $26.00 \pm 0.00$  mm). For fungal strains, *Citrus sinensis* peel essential oil showed the strongest antifungal efficacy against *Cryptococcus neoformans* ( $34.33 \pm 0.52$  mm) and *Candida albicans* ( $39.67 \pm 1.63$  mm). *Citrus sinensis* and *Citrus clementina* peel essential oils exhibited mostly bactericidal and fungicidal effects against the majority of tested strains.

**Conclusions.** Moroccan citrus peel essential oils exhibited strong antimicrobial properties, highlighting their potential as natural substances and supporting their valorization in biomedicine and industrial applications.

**Keywords:** citrus peel, essential oils, antibacterial activity, antifungal activity, natural products, public health

### INTRODUCTION

Bacterial and fungal infectious diseases constitute a serious public health problem worldwide [1]. Bacterial infections cause around 12.5% of all deaths worldwide, whereas fungal infections afflict about 150 million people each year and cause about 1.7 million deaths [2, 3]. Additionally, it has been shown that a number of harmful microbes have become resistant to standard antibiotic treatments [4]. Consequently, plant-based treatments are becoming more and more well-liked as organic suppliers of antibacterial qualities. The *Rutaceae* family includes *Citrus* species [5], which

rank among the most cultivated and widely consumed crops [6-8]. Of the 140 million tons of citrus produced annually worldwide [9], around 18% is used for industrial processing, like juice production [8]. Pulp, seeds, and peels are among the substantial amounts of by-products produced by this extensive use [8]. Citrus peels, which comprise between 50 and 60 percent of raw fruits, are the most common residue [10]. Because these byproducts are frequently underutilized, their accumulation raises environmental issues [11].

Nonetheless, essential oils, phenolic acids, carotenoids, and flavonoids are among the natural and active compounds that can be found in citrus

**Corresponding author:** Loubna Ouahmane, Laboratory of Analysis, Modeling, Engineering, Natural Substances and Environment, Polydisciplinary Faculty of Taroudant, Ibn-Zohr University, 83000 Taroudant, Morocco; email: loubna.ouahmane.62@edu.uiz.ac.ma

This article is available in Open Access model and licensed under a Creative Commons Attribution-Non Commercial 4.0 International License (CC BY-NC) (<https://creativecommons.org/licenses/by-nc/4.0/>)

Publisher: National Institute of Public Health NIH - National Research Institute

fruit peels [12-14]. More than 400 chemicals make up citrus essential oils (CEOs), which are complex combinations mostly consisting of monoterpenes, flavonoids, sesquiterpenes, coumarins, and oxygenated derivatives [14, 15]. Limonene, a cyclic monoterpene (C<sub>10</sub>H<sub>16</sub>) [16], is typically the most prevalent of these compounds [17]. CEOs' chemical makeup, especially that of d-limonene and flavonoids, is linked to many of the biological properties, notably antifungal, antioxidant, antibacterial, and anti-inflammatory activities [18-20].

However, notwithstanding the increasing amount of research on citrus essential oils, a gap remains in comparative information on the antimicrobial effects, particularly about the high value of these by-products for medical purposes. This work aims to clarify the antibacterial and antifungal effects of citrus peel essential oils from four Moroccan varieties and to encourage the value-adding of citrus peel by-products as a natural and environmentally friendly substitute with potential uses in the food and health sectors by showcasing their bioactive potential.

## MATERIALS AND METHODS

### Materials

#### *Biological material*

The collected plant material selected for the present work consisted of the peels of citrus fruits from four varieties: *Citrus limon* (lemon), *Citrus clementina* (clementine), *Citrus sinensis* (orange), and *Citrus aurantium* (bitter orange). The fruits were purchased from a local market in Taroudant City, Morocco. Only mature, healthy fruits free from visible defects were selected. The plant material was botanically identified by the research team of the Laboratory of Natural Substances and Environment at Ibn Zohr University.

#### *Microorganisms*

For this study, eight bacterial and two fungal strains were chosen. The bacterial panel included four Gram-positive (*Bacillus* sp. CIP104717, *Staphylococcus aureus* ATCC25923, *Listeria monocytogenes* ATCC19144, and *Enterococcus faecalis* ATCC19433) and four Gram-negative (*Pseudomonas* sp., *Pseudomonas fluorescens* CAN 228-1, *Escherichia coli* CIP54127, and *Salmonella* Typhimurium ATCC14028). *Candida albicans* CIP88465 and *Cryptococcus neoformans* ATCC11576 were the fungal strains used to assess antifungal activity. Reference strains were obtained from recognized collections, including the American Type Culture Collection (ATCC) and the Pasteur Institute's collection (CIP). However, *Pseudomonas* sp., and *Pseudomonas fluorescens* CAN 228-1 were obtained from the laboratory microbial collection and were

previously identified using standard microbiological methods. Yeast strains were cultivated on Sabouraud dextrose agar (SDA), while bacterial strains were cultivated on Luria-Bertani (LB) agar.

### Methods

#### *Essential oils extraction*

Fresh citrus peels served for essential oils extraction. The fruits were thoroughly washed with distilled water to remove impurities. The peels were carefully separated and cut into small pieces. A Clevenger-type apparatus was used for 3 h of hydrodistillation. A total of 600 g of fresh peels was used for each essential oil. The essential oils were dehydrated using anhydrous sodium sulfate [2]. The following equation was employed to measure the yields of extracted citrus peel essential oils:

$$\text{Oil yield (\%)} = \frac{\text{Volume of the extracted essential oil (mL)}}{\text{Fres peel weight (g)}} \times 100$$

Based on the available scientific data, it can be concluded that, unlike hydrodistillation/steam distillation methods for extracting essential oils, cold pressing may imply a high risk of transferring lipophilic fungicides into the oils. For these reasons, the observed antimicrobial and antifungal activity of essential oils obtained by hydrodistillation is mainly due to their natural properties.

#### *Antibacterial and antifungal activities*

The antibacterial potential was evaluated using the disk-diffusion assay [21]. Yeast Morphology Agar (YMA) (Difco, USA) was used for antifungal experiments, and Mueller-Hinton Agar (MHA) (Difco, USA) was used to measure antibacterial activity. Suspensions of bacteria and yeast were prepared from 24-hour cultures. The sampled colonies were transferred to 9 mL of API NaCl 0.85% medium (bioMérieux, France) and adjusted to 10<sup>6</sup> CFU/mL for bacteria and 10<sup>4</sup> CFU/mL for yeasts. Sterile paper discs (6 mm) impregnated with 15 µL of citrus peel essential oils were aseptically placed on the surface of agar after the standardized inocula were dispersed onto the relevant agar media. A pre-diffusion step was carried out at 4°C for 2 h to facilitate uniform diffusion of the essential oils into the agar before microbial growth. The plates were incubated for 24 h at 37°C for bacteria and 48 h at 28°C for yeasts. For antifungal assays, fluconazole and econazole (30 µg/disc) were the positive controls, while chloramphenicol, penicillin, and gentamicin (30 µg/disc) served as antibacterial controls. Antifungal and antibacterial activities were expressed as the mean diameter of the

zone of inhibition (ZI) (mm), and all experiments were conducted in triplicate (Table 1).

#### *Minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration (MIC) values of citrus peel essential oils were quantified using the broth microdilution technique, which is performed in sterile 96-well microplates according to Chebaibi et al. [22]. Two-fold serial dilutions of each essential oil were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA) at concentrations between 25 and 0.097 mg/mL. Each well contained culture medium (Sabouraud dextrose broth for yeasts and Mueller-Hinton broth (Difco, USA) for bacteria), essential oil solution, and a standardized microbial inoculum ( $10^8$  CFU/mL and  $10^6$  CFU/mL for yeast and bacteria, respectively). Negative control wells were composed of DMSO and culture medium, whereas wells designed for growth control contained medium and inoculum without essential oil. All assays were performed in triplicate. Microplates were incubated at 37°C for 24 h for bacteria and 48 h for yeasts. Following incubation, 0.04% resazurin solution was added, followed by an additional 2-hour incubation. The MIC is defined as the lowest essential oil concentration that does not cause a color shift, signifying the inhibition of microbial growth.

#### *Minimum fungicidal and bactericidal concentration (MFC and MBC)*

The MFC and MBC were identified as described by Chebaibi et al. [22]. After MIC determination, aliquots (10 µL) from wells showing no visible microbial growth were subcultured onto Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for yeasts. The incubation was carried out at 28 °C for 48 h for yeasts and at 37°C for 24 h for bacteria. The MFC and MBC were defined as the lowest concentration of essential oil at which no microbial growth was observed on agar plates. All assays were performed in triplicate.

#### *Statistical analysis*

All experiments are expressed in triplicate, and results are expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate differences between the tested citrus peel essential oils. A p-value  $< 0.05$  was considered statistically significant.

## RESULTS

### **The yield of fresh citrus peel studied**

The citrus fruits of the four studied varieties (*Citrus sinensis*, *Citrus limon*, *Citrus clementina*, and *Citrus aurantium*) were collected between November 2023 and January 2024. Hydrodistillation was utilized

to extract essential oils from fresh peels. The yield of essential oils varied among the studied varieties, ranging from 0.4% to 1.0%. *Citrus clementina* had the lowest output (0.4%), followed by *Citrus sinensis* (0.6%) and *Citrus limon* (0.8%), whereas *Citrus aurantium* peel essential oil exhibited the highest yield (1.0%). In addition, the physical properties of the extracted citrus peel essential oils, such as colour, viscosity, and scent, varied noticeably.

### **Antimicrobial activity assay**

#### *Antibacterial activity*

Eight bacterial strains, including four Gram-positive and four Gram-negative bacteria, were used to test the four citrus peel essential oils' (CPEOs') *in vitro* antibacterial activity (Table 1). The *Citrus* species and the bacterial strain under examination both affected the antibacterial activity. *Citrus limon* showed the lowest antibacterial activity among the essential oils, whereas *Citrus sinensis* was characterized by the highest activity against most bacterial strains. All the evaluated essential oils showed minimal sensitivity to *Listeria monocytogenes*. Several bacterial strains, including *Pseudomonas* sp., *Pseudomonas fluorescens*, *Escherichia coli*, *Bacillus* sp., *Staphylococcus aureus*, and *Enterococcus faecalis*, were highly susceptible to *Citrus sinensis* and *Citrus clementina* peel essential oils. *Citrus aurantium* essential oil showed a modest level of antibacterial activity, with the strongest inhibitory impact on *Pseudomonas* sp. Conversely, the only bacterial strain that was susceptible to the essential oil found in *Citrus limon* peel was *Escherichia coli*. Significant differences were observed between the tested citrus peel essential oils against *Escherichia coli* ( $p < 0.05$ ).

#### *Antifungal activity*

Applying the agar disk-diffusion technique, the *in vitro* antifungal activity of the four citrus peel essential oils (CPEOs) was assessed against two pathogenic yeasts, *Candida albicans* CIP88465 and *Cryptococcus neoformans* ATCC11576. The inhibition zone diameters obtained are presented in Table 2. Overall, *Citrus sinensis*, *Citrus clementina*, and *Citrus aurantium* essential oils exhibited stronger antifungal activity than *Citrus limon*. Among the tested oils, the essential oil of *Citrus sinensis* demonstrated the strongest antifungal activity against both fungal strains. In general, *Candida albicans* was more sensitive to the tested CPEOs than *Cryptococcus neoformans*, as indicated by the larger inhibition zone diameters observed across all essential oils. Statistical analysis using one-way ANOVA revealed highly significant differences between the tested groups against *Candida albicans* ( $p < 0.001$ ).

Table 1. *In vitro* antibacterial activity of citrus peel essential oils (CPEOs) and reference antibiotics against the tested bacterial strains

CPEOs	<i>Citrus limon</i>			<i>Citrus clementina</i>			<i>Citrus sinensis</i>			<i>Citrus aurantium</i>			Antibiotic (ZI ± SD, mm)		
	Inhibition zone <sup>a</sup> (mm)	Min	Max	Inhibition zone <sup>a</sup> (mm)	Min	Max	Inhibition zone <sup>a</sup> (mm)	Min	Max	Inhibition zone <sup>a</sup> (mm)	Min	Max	Pen	Chlor	Genta
Gram-positive bacteria	<i>Bacillus</i> sp. CIP104717	ND	ND	14.50 ± 0.71	14.00	15.00	24.50 ± 0.71	24.00	25.00	ND	ND	ND	22.33 ± 0.44	ND	ND
	<i>Staphylococcus aureus</i> ATCC25923	10.00 ± 0.00	10.00	13.00 ± 1.41	12.00	14.00	21.00 ± 2.65	19.00	24.00	11.50 ± 0.71	11.00	12.00	20.00 ± 0.67	ND	ND
	<i>Listeria monocytogenes</i> ATCC19144	9.67 ± 1.15	9.00	8.00 ± 1.00	7.00	9.00	9.00 ± 1.00	8.00	10.00	7.33 ± 0.58	7.00	8.00	ND	28.00 ± 0.67	ND
	<i>Enterococcus faecalis</i> ATCC19433	6.00 ± 1.00	5.00	17.00 ± 1.00	16.00	18.00	19.00 ± 1.00	18.00	20.00	5.33 ± 1.53	4.00	7.00	ND	15.16 ± 0.22	ND
	<i>Escherichia coli</i> CIP54127	12.00 ± 1.41	11.00	18.00 ± 6.24	13.00	25.00	25.33 ± 1.53	24.00	27.00	11.00 ± 0.00	11.00	11.00	ND	ND	32.66 ± 0.44
Gram-negative bacteria	<i>Pseudomonas</i> sp.	ND	ND	23.33 ± 3.21	21.00	27.00	33.33 ± 2.08	31.00	35.00	15.50 ± 0.71	15.00	16.00	ND	ND	42.33 ± 0.46
	<i>Pseudomonas fluorescens</i> CAN 228-1	ND	ND	19.00 ± 1.41	18.00	20.00	26.00 ± 0.00	26.00	26.00	11.50 ± 0.71	11.00	12.00	ND	ND	44.00 ± 0.68
	<i>Salmonella</i> Typhimurium ATCC14028	4.33 ± 0.58	4.00	14.00 ± 1.00	13.00	15.00	16.00 ± 1.00	15.00	17.00	4.33 ± 0.58	4.00	5.00	ND	ND	26.16 ± 0.55

<sup>a</sup> – Data are presented as mean ± standard deviation (n = 3); ND – not determined; Pen – penicillin; Chlor – chloramphenicol; Genta – gentamicin. The measurement scale was as follows: no inhibition: < 9 mm; moderate inhibition: 9-12 mm; strong inhibition: 13-15 mm; very strong inhibition: > 15 mm.

Table 2. *In vitro* antifungal activity of citrus peel essential oils (CPEOs) and reference antifungal agents against the tested fungal strains

CPEOs	<i>Candida albicans</i> CIP88465			<i>Cryptococcus neoformans</i> ATCC11576		
	Inhibition zone (mm) <sup>a</sup>	Minimum	Maximum	Inhibition zone (mm) <sup>a</sup>	Minimum	Maximum
<i>Citrus limon</i>	13.33 ± 0.52	13.00	14.00	10.33 ± 0.82	9.00	11.00
<i>Citrus clementina</i>	27.17 ± 2.04	23.00	28.00	27.33 ± 1.03	26.00	28.00
<i>Citrus sinensis</i>	39.67 ± 1.63	37.00	42.00	34.33 ± 0.52	34.00	35.00
<i>Citrus aurantium</i>	28.50 ± 1.05	27.00	30.00	26.17 ± 1.60	23.00	27.00
Fluconazole	24.83 ± 2.40	20.00	26.00	25.17 ± 2.56	20.00	27.00
Econazole	40.33 ± 2.88	38.00	44.00	38.67 ± 0.52	38.00	39.00

<sup>a</sup> – Data are presented as mean ± standard deviation (n = 3).

### Minimum inhibitory concentration (MIC), minimum fungicidal (MFC) concentration, and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC), fungicidal (MFC), and bactericidal (MBC) concentrations of citrus peel essential oils were determined, and MFC/MIC or MBC/MIC ratios were used to evaluate fungicidal and bactericidal effects (Tables 3 and 4). An MFC/MIC ≤ 4 or MBC/MIC indicates a fungicidal or bactericidal activity, whereas

a ratio > 4 demonstrates a fungistatic or bacteriostatic effect [22].

For Gram-positive bacteria, MIC results varied between 3.12 and 25 mg/mL. *Citrus sinensis* and *Citrus clementina* peel essential oils exhibited bactericidal activity against most tested strains, whereas *Citrus aurantium* did not show bactericidal activity within the tested concentration range. *Enterococcus faecalis* was the most resistant strain, showing elevated MBC/MIC ratios. For Gram-negative bacteria, *Citrus sinensis*

Table 3. *In vitro* MIC, MBC, and MBC/MIC values of the selected citrus peel essential oils against tested bacterial strains

CPEOs	<i>Citrus clementina</i>			<i>Citrus sinensis</i>			<i>Citrus aurantium</i>			
	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	
Gram-positive bacteria	<i>Bacillus</i> sp. CIP104718	6.25	25.00	4.00	3.12	12.50	4.00	ND	ND	ND
	<i>Staphylococcus aureus</i> ATCC25923	12.50	25.00	2.00	12.50	25.00	2.00	25.00	> 25	> 1
	<i>Enterococcus faecalis</i> ATCC19434	3.12	25.00	8.1	0.78	12.50	16.02	ND	ND	ND
Gram-negative bacteria	<i>Escherichia coli</i> CIP54128	12.50	12.50	1.00	6.25	12.50	2.00	25.00	> 25	> 1
	<i>Pseudomonas</i> sp.	12.50	25.00	2.00	6.25	25.00	4.00	ND	ND	ND
	<i>Pseudomonas fluorescens</i> CAN 228-1	6.25	25.00	4.00	6.25	25.00	4.00	ND	ND	ND
	<i>Salmonella</i> Typhimurium ATCC14028	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND – not determined

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the tested citrus peels essential oils (CPEOs) against the selected fungal strains

CPEOs	<i>Candida albicans</i> CIP88465			<i>Cryptococcus neoformans</i> ATCC11576		
	MIC (mg/mL)	MFC (mg/mL)	MFC/MIC	MIC (mg/mL)	MFC (mg/mL)	MFC/MIC
<i>Citrus limon</i>	25.00	25.00	1.00	3.12	25.00	8.01
<i>Citrus clementina</i>	3.12	6.25	2.00	1.56	12.50	8.01
<i>Citrus sinensis</i>	3.12	6.25	2.00	6.25	6.25	1.00
<i>Citrus aurantium</i>	25.00	25.00	1.00	3.12	12.50	4.01
Fluconazole	0.25	0.25	1.00	0.25	0.25	1.00
Econazole	0.06	0.12	1.93	0.06	0.12	1.93

and *Citrus clementina* peel demonstrated bactericidal activity against most strains, while *Citrus aurantium* exhibited a bacteriostatic effect against *Escherichia coli*.

Regarding fungal strains, all tested essential oils exhibited a fungicidal effect against *Candida albicans*. In contrast, against *Cryptococcus neoformans*, *Citrus limon*, *Citrus clementina*, and *Citrus aurantium* exhibited fungistatic effects, whereas *Citrus sinensis* showed a fungicidal effect.

## DISCUSSION

Four Moroccan citrus peel essential oils were tested for antimicrobial effectiveness against two yeast species and eight bacterial strains. Although minor differences were noted, the citrus peel essential oil yields found in this study are largely in line with those found in earlier studies. *Citrus limon* essential oil production (0.8%) was similar to what Abdu et al. [23] reported. On the other hand, *Citrus clementina* yielded a little smaller amount than Boudries et al. [21]. The yield of *Citrus aurantium* peel essential oil was greater than what Boukhenoufa et al. [15] had reported. In a similar vein, *Citrus sinensis* peel essential oil output was higher than that of Naveed et al. [24]. The harvesting period, soil properties, climate, water availability, plant genotype, and extraction technique are some of the variables that may be responsible for the well-documented variations in essential oil yields [21, 22, 25]. This study's *in vitro* antibacterial activity is in line with results on citrus peel essential oils that have already been reported. *Citrus sinensis*'s broad-spectrum antibacterial effectiveness against *Staphylococcus aureus* and *Escherichia coli* has been confirmed by numerous studies [26-28]. Previous studies have also documented the sensitivity of *Enterococcus faecalis* to *Citrus sinensis*, which supports the findings of this work [26]. *Citrus limon* peel essential oil has also been shown in a number

of studies to demonstrate a mild antibacterial effect against *E. coli*, *S. aureus* and *Listeria monocytogenes*, which is similar to the activity found in our study [27, 29, 30]. In line with the study's findings of a moderate inhibitory impact, the essential oil of *Citrus clementina* has also been demonstrated to possess an inhibitory effect against *E. coli* and *S. aureus* [21]. Strong antibacterial action against *S. aureus* has also been demonstrated for *Citrus aurantium* peel essential oil, particularly when diluted in dimethyl sulfoxide (DMSO) [31]. Citrus peel essential oils' antibacterial activity and mode of action are influenced by their chemical composition [21]. The mechanism of action, which is frequently connected to the permeability of the bacterial cell wall and membrane, explains the reduced susceptibility observed in *Enterococcus faecalis* [32]. The *in vitro* antifungal effectiveness of the current study is consistent with previous investigations that described *Candida albicans*' vulnerability to citrus essential oils. Numerous studies have demonstrated the strong antifungal effect of *Citrus sinensis* essential oil against *Candida albicans*, exhibiting an inhibition zone (ZI) between 29 and 55 mm [26, 33]. The inhibitory zone identified in this study, which is within this range, validates the essential oil's antifungal properties. Similar to the current investigation, Boudries et al. [21] found a pronounced antifungal effect of *Citrus clementina* peel essential oil against *C. albicans* (ZI = 27.63 mm). The mild antifungal activity seen in this investigation is comparable to what was reported by Abdu et al. [23] for *Citrus limon* against the same yeast (ZI = 18 mm). Furthermore, Nidhi et al. [34] showed that *Candida albicans* was sensitive to the essential oil of *Citrus aurantium* peel, which confirmed the antifungal effect found in this study.

There is a significant dearth of published information about citrus peel essential oils' antifungal activity against *Cryptococcus neoformans*, in contrast to the many studies that have examined their

effectiveness against *Candida albicans*. An important contribution that emphasises the uniqueness and added value of this work is the robust antifungal activity that was found in the current investigation against this opportunistic pathogenic yeast. *Citrus sinensis* and *Citrus clementina* essential oils showed superior bactericidal and fungicidal activity against most of the tested bacteria, as evidenced by the calculation of MIC, MBC, and MFC values. Both the action of the primary ingredients, like monoterpenes, and potential synergistic interactions between the major and small components of the essential oils may be responsible for these effects [21].

## CONCLUSION

The present study demonstrated that the four Moroccan citrus peel essential oils represent a valuable source of antibacterial and antifungal agents. *Citrus sinensis* and *Citrus clementina* exhibited the strongest antimicrobial activity, showing predominantly bactericidal and fungicidal effects against most of the tested strains, whereas *Citrus limon* and *Citrus aurantium* displayed moderate antimicrobial activity. Importantly, this research offers fresh perspectives on the effectiveness of citrus peel essential oils as antifungals against *Cryptococcus neoformans*, an opportunistic pathogen that has been rarely investigated, which constitutes a key originality of this work. Notably, *Citrus sinensis* peel essential oil exhibited a fungicidal effect against this yeast. Overall, these results demonstrate the antimicrobial properties of citrus peel by-products as natural antimicrobial agents and support their potential as alternatives to conventional treatments such as commonly used antibiotics (e.g., chloramphenicol, penicillin, and gentamicin) and antifungal agents (e.g., fluconazole, econazole). They may also serve as promising resources for developing new pharmaceutical agents for global health applications. Additionally, the antibacterial activity of Moroccan citrus essential oils appears to be comparable to that observed in citrus oils from other regions of the world. Nonetheless, variations in the chemical composition of the essential oils, geographical origin, and climatic conditions may account for small differences in activity.

## Acknowledgments

This work was supported by the National Center for Scientific and Technical Research (CNRST) under the "PhD-Associate Scholarship-PASS" program, which is gratefully acknowledged by the authors.

## Conflict of interest

No conflict of interest is declared by the authors.

## REFERENCES

1. Nii-Trebi NI. Emerging and Neglected Infectious Diseases: Insights, Advances, and Challenges. *Biomed Res Int.* 2017;2017:5245021. doi: 10.1155/2017/5245021.
2. Olatunya AM, Olatunya OS, Hassan GF, Adeyemi LA, Oyelami OA, Akintayo ET. Antimicrobial activities of essential oils from three species of citrus fruits against six infectious tropical bacteria: implications for the care and control of common bacterial tropical diseases in developing countries. *Discov Appl Sci.* 2024;6:209. doi: 10.1007/s42452-024-05813-5.
3. Kainz K, Bauer MA, Madeo F, Carmona-Gutierrez D. Fungal infections in humans: the silent crisis. *Microb Cell.* 2020;7(6):143-145. doi: 10.15698/mic2020.06.718.
4. Hossain CM, Ryan LK, Gera M, Choudhuri S, Lyle N, Ali KA, et al. Antifungals and drug resistance. *Encyclopedia.* 2022;2(4):1722-1737. doi: 10.3390/encyclopedia2040118.
5. Brah AS, Armah FA, Obuah C, Akwetey SA, Adokoh CK. Toxicity and therapeutic applications of citrus essential oils (CEOs): a review. *Int J FOOD Prop.* 2023;26(1):301-326. doi: 10.1080/10942912.2022.2158864.
6. Ashmawy NS, Nilofar N, Zengin G, Eldahshan OA. Metabolic profiling and enzyme inhibitory activity of the essential oil of *Citrus aurantium* fruit peel. *BMC Complement Med Ther.* 2024;24(1):262. doi: 10.1186/s12906-024-04505-2.
7. Dang NH, Nhung PH, Mai Anh BT, Thuy DTT, Minh CV, Dat NT. Chemical Composition and  $\alpha$ -Glucosidase Inhibitory Activity of Vietnamese Citrus Peels Essential Oils. *J Chem.* 2016;2016:6787952. doi: 10.1155/2016/6787952.
8. Anticono M, Blesa J, Frigola A, Esteve MJ. High Biological Value Compounds Extraction from Citrus Waste with Non-Conventional Methods. *Foods.* 2020;9(6):811. doi: 10.3390/foods9060811.
9. Anastasopoulou E, Graikou K, Ziogas V, Ganos C, Calapai F, Chinou I. Chemical Profiles and Antimicrobial Properties of Essential Oils from Orange, Pummelo, and Tangelo Cultivated in Greece. *Horticulturae.* 2024;10(8):792. doi: 10.3390/horticulturae10080792.
10. da Costa JS, Maranduba HL, de Sousa Castro S, de Almeida Neto JA, Rodrigues LB. Environmental performance of orange citrus waste as raw material for pectin and essential oil production. *Food Bioprod Process.* 2022;135:165-177. doi: 10.1016/j.fbp.2022.07.008.
11. Agarwal P, Sebghatollahi Z, Kamal M, Dhyani A, Shrivastava A, Singh KK, et al. Citrus Essential Oils in Aromatherapy: Therapeutic Effects and Mechanisms. *Antioxidants (Basel).* 2022;11(12):2374. doi: 10.3390/antiox11122374.
12. Martinidou E, Michailidis M, Ziogas V, Masuero D, Angeli A, Moysiadis T, et al. Comparative Evaluation of Secondary Metabolite Chemodiversity of Citrus Genebank Collection in Greece: Can the Peel be More than Waste? *J Agric Food Chem.* 2024;72(1):9019-9032. doi: 10.1021/acs.jafc.4c00486.

13. Munir H, Yaqoob S, Awan KA, , Imtiaz A, Naveed H, Ahmad N, et al. Unveiling the Chemistry of Citrus Peel: Insights into Nutraceutical Potential and Therapeutic Applications. *Foods*. 2024;13(11):1681. doi: 10.3390/foods13111681.
14. Singh B, Singh JP, Kaur A, Yadav MP. Insights into the chemical composition and bioactivities of citrus peel essential oils. *Food Res Int*. 2021;143:110231. doi: 10.1016/j.foodres.2021.110231.
15. Boukhenoufa A, Tir Touil Meddah A, Meddah B, Gabaldon JA, Sonnet P. Comparative study of *Artemisia herba alba* Asso and *Citrus aurantium* essential oils. *J Microbiol Biotechnol Food Sci*. 2019;9(3):622-627. doi: 10.15414/jmbfs.2019/20.9.3.622-627.
16. Battista F, Remelli G, Zanzoni S, Bolzonella D. Valorization of residual orange peels: limonene recovery, volatile fatty acids, and biogas production. *ACS Sustain Chem Eng*. 2020;8(17):6834-6843. doi: 10.1021/acssuschemeng.0c01735.
17. Ferrer V, Paymal N, Costantino G, Paoli M, Quinton C, Tomi F, et al. Correspondence between the compositional and aromatic diversity of leaf and fruit essential oils and the pomological diversity of 43 sweet oranges (*Citrus x aurantium* var. *sinensis* L.). *Plants (Basel)*. 2023;12(5):990. doi: 10.3390/plants12050990.
18. Bhandari DP, Chaudhary P, Upadhyaya SR, Ranjitkar R, Satyal R, Adhikari A, et al. Chemical variability, antioxidant and larvicidal efficacy of EOs from *Citrus sinensis* (L.) Osbeck peel, leaf, and flower. *Horticulturae*. 2024;10(6):566. doi: 10.3390/horticulturae10060566.
19. Ibrahim FM, Mohammed RS, Abdelsalam E, Ashour WES, Magalhães D, Pintado M, El Habbasha ES. Egyptian citrus essential oils recovered from lemon, orange, and mandarin peels: phytochemical and biological value. *Horticulturae*. 2024;10(2):180. doi: 10.3390/horticulturae10020180.
20. Tundis R, Xiao J, Sanches Silva , Carreiró F, Loizzo MR. Health-promoting properties and potential application in the food industry of *Citrus medica* L. and *Citrus × clementina* Hort. Ex Tan. essential oils and their main constituents. *Plants (Basel)*. 2023;12(5):991. doi: 10.3390/plants12050991.
21. Boudries H, Loupassaki S, Ladjal Ettoumi Y, Souagui S, Bachir Bey M, Nabet N, et al. Chemical profile, antimicrobial and antioxidant activities of *Citrus reticulata* and *Citrus clementina* (L.) essential oils. *Int Food Res J*. 2017;24(4):1782-1792. doi: 10.1007/s11694-017-9558-8.22.
22. Chebaibi A, Marouf Z, Rhazi-Filali F, Fahim M, Ed-Dra A. Evaluation of the antimicrobial activity of essential oils from seven medicinal plants harvested in Morocco. *Phytotherapy*. 2016;14(6):355-362. doi: 10.1007/s10298-015-0996-1.
23. Abdu KM, Erahioui R, Zahidi A, Khedid K, Ibn Ahmed S. Evaluation of antifungal activity of lemon (*Citrus limon*) in Morocco. *Egypt J Chem*. 2021;64(8):4183-4190. doi: 10.21608/EJCHEM.2021.54730.3143.
24. Naveed R. Antimicrobial activity of bioactive components of essential oils from *Citrus sinensis* against important pathogens. *Int Food Res J*. 2021;28(1):189-198. doi: 10.47836/ifrj.28.1.19.
25. Bouftila B, Abdessadek M, Ben-Saghroune H, Sbihi K, Ouahmane L, Amri O, et al. Antimicrobial activities of essential oils of plant species from Morocco. *Rocz Panstw Zakl Hig*. 2024;75(2):117-123. doi: 10.32394/rpzh.2024.0297.
26. Obidi OF, Adelowotan AO, Ayoola GA, Johnson OO, Hassan MO, Nwachukwu SCU. Antimicrobial activity of orange oil on selected pathogens. *Int J Biotechnol*. 2013;2(6):113-122.
27. Marithangam A, Sarojini VP, Al Farraj DA, Elshikh MS, Arokijaraj S. Essential oils from citrus fruit peels to control foodborne bacteria in fresh-cut guava fruits. *BioResources*. 2025;20(2):2587-2608. doi: 10.15376/biores.20.2.2587-2608.
28. Anwar T, Qureshi H, Fatima A, Sattar K, Albasher G, Kamal A, et al. *Citrus sinensis* peel oil extraction and evaluation as an antibacterial and antifungal agent. *Microorganisms*. 2023;11(7):1662. doi: 10.3390/microorganisms11071662.
29. Gupta C. Chemical composition of fruit peels essential oil from *Citrus* species and their antimicrobial efficacy as biopreservatives. *Adv Food Technol Nutr Sci*. 2022;8(1):29-35. doi: 10.17140/AFTNSOJ-8-177.
30. Kostova I, Lasheva V, Georgieva D, Damyanova S, Stoyanova A, Stefanov S, et al. Antimicrobial active packaging paper based on dill weed essential oil. *Cellul Chem Technol*. 2020;54(3-4):347-354. doi: 10.35812/CELLULOSECHEMTECHNOL.2020.54.35.
31. Punitha VN, Vijayakumar S, Nilavukkarasi M, Vidhya E, Praseetha PK. Fruit peels that unlock curative potential: determination of biomedical application and bioactive compounds. *South Afr J Bot*. 2022;150:1051-1060. doi: 10.1016/j.sajb.2022.09.022.
32. Hacib H, Lakache Z, Aliboudhar H, Laassami A, Tounssi H, Hamil S, et al. Chemical composition and antioxidant and antibacterial properties of the essential oils extracted from *Citrus sinensis* peels by hydrodistillation and microwave methods. *Environ Sci Pollut Res*. 2024;31(28):40748-40757. doi: 10.1007/s11356-023-31526-0.
33. Dehury S, Priyadarsini P, Nanda A, Dubey D, Swain SK, Samantaray B, et al. *In silico* molecular docking, antimicrobial and anti-inflammatory activities of bioactive fractions of *Citrus sinensis* (L.) Osbeck. peel against oral pathogens in dental caries. *Plant Sci Today*. 2024;11(4):373-384. doi: 10.14719/pst.2922.
34. Nidhi P, Rolta R, Kumar V, Dev K, Sourirajan A. Synergistic potential of *Citrus aurantium* L. essential oil with antibiotics against *Candida albicans*. *J Ethnopharmacol*. 2020;262:113135. doi: 10.1016/j.jep.2020.113135.

Received: 28.03.2026

Revised: 11.06.2026

Accepted: 16.06.2026