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ARTICLE

Antimicrobial Potential of *Citrus limon* Peel Essential Oil from Morocco: A Sustainable Approach to Citrus Waste Valorization

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ABSTRACT

Citrus peel residues are among the most abundant agro-industrial by-products, and their sustainable valorization offers a promising strategy to mitigate environmental impacts while generating value-added products for agriculture and health.

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In this study, approximately 130 lemon fruits were harvested from three representative orchards in the Pre-Rif region (Taza, Morocco) in March 2022. In each orchard, 5–10 trees were randomly selected, and 3–5 mature and healthy fruits were collected per tree. The peels were separated, washed, and subjected to hydrodistillation for essential oil (EO) extraction. The EO was chemically characterized by gas chromatography–mass spectrometry (GC–MS), following authentication of plant material through morphological and molecular identification. Twenty volatile compounds were identified, with D-limonene (46.89%) as the major constituent, followed by citral (8.32%), benzene (ethoxymethyl)- (7.86%), α-pinene (6.89%), cyclopentane bromo- (6.39%), and 1, 2-bis(3-cyclohexenyl)ethylene (5.33%). Biological assays revealed strong broad-spectrum antimicrobial activity against clinically relevant pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Candida albicans*. The lowest Minimum Inhibitory Concentration (MIC) was recorded for *C. albicans* (1.66 mg/mL), while both *S. aureus* and *P. aeruginosa* showed pronounced bactericidal responses (MIC = MBC = 6.63 mg/mL). These findings demonstrate the potential of *C. limon* EO as a natural antimicrobial agent. By converting citrus peel waste into high-value bioproducts, this study highlights both environmental and socio-economic benefits, fostering eco-friendly biopesticides suitable for Integrated Pest Management (IPM) and reinforcing the circular bioeconomy in citrus-producing regions.

Keywords: *Citrus limon*; Essential Oil; GC–MS; Antimicrobial Activity; Sustainable Valorization

1. Introduction

Citrus fruits (Rutaceae), such as sweet orange (*C. sinensis*), lemon (*C. limon*), mandarin (*C. reticulata*), grapefruit (*C. paradisi*), and bergamot (*C. bergamia*), are some of the most widely grown fruit crops globally [1]. Over 140 countries grow citrus, providing over 150 million of citrus crops every year, and citrus, in particular, is not only an essential part of the worldwide horticulture but it is an important economic crop for world trade [2,3]. Apart from the economic importance of citrus fruits, their nutritional qualities and health-promoting properties as a source of bioactive compounds that can contain flavonoids, carotenoids, limonoids, pectins, and essential oils (EOs), which have been documented to have antioxidant, antimicrobial, anti-inflammatory, and anticancer properties [4,5]. They are the basis for many of the uses found in food, pharmaceutical, cosmetic, and agricultural industries [6.7]. Therefore, citrus with this range of documented uses is one of the best researched groups of fruit out there [8].

While citrus production and processing are important contributors to the global economy, they also create enormous amounts of residues consisting of mainly peels, seeds and pulp, which can account for 50–60% of the total fruit mass produced [9]. This translates to more than 60 million tons of organic waste created globally every year, a large proportion of which is not adequately being utilized. If not disposed of properly, the biomass can be a source of environmental burdens–such as greenhouse gas emissions, leachate pollution and public health risks [10]. Utilizing citrus by-products in the production of value-added products–such as bioactive extracts, biosorbents, nanomaterials, or biofertilizers–offers a potential solution to waste reduction and promotes circular bioeconomy approaches [11]. According to these environmental issues, today's agriculture also faces increased challenges as a consequence of the dependence on synthetic pesticides and other chemicals [12]. These chemicals may be effective short-term, but have harmful environmental and health impacts such as pollution of soil and water, loss of biodiversity, and resistance in pests and pathogen populations [13]. When pests and disease populations develop resistance it reduces our crop productivity and thus threatens food security, and encourages the need to transition to more sustainable alternatives [14]. Citrus peels are particularly high in volatile terpenes (D-limonene, citral and α-pinene) known for their antimicrobial, insecticidal and antioxidant properties [15]. Many studies have reported the effectiveness of citrus essential oils (EOs) against important agricultural pests such as *Spodoptera littoralis*, *Tuta absoluta*, *Bemisia tabaci*, and *Aphis* spp., as well as against stored-product insects including *Sitophilus* spp. and *Tribolium castaneum* [16]. Furthermore, citrus EOs exhibited inhibitory activity against plant pathogenic fungi such as *Botrytis cinerea*, *Alternaria alternata*, and *Fusarium oxysporum*. These bioactivities have been demonstrated in studies conducted across various countries, including Brazil[17], Tunisia[18], Iran[19], India[20], Ethiopia[21], China[22], Malaysia[23,24], Thailand[25,26], Sri Lanka[27], and Korea[28], confirming the global relevance of citrus EO applications in pest and disease management. These studies support the potential of citrus EOs as environmentally friendly and safer alternatives to synthetic biopesticides as part of an integrated pest management program[29,30]. Nevertheless, the physiochemical composition and biological activity of *C. limon* essential oils vary greatly depending on cultivar genetics, pedoclimatic conditions, harvest maturity, and extraction methods[31]. In Morocco–one of the largest citrus-producing countries in the Mediterranean basin–the Pre-Rif region represents a unique ecological niche due to its specific pedological and climatic conditions, which may modulate the phytochemical profile of lemon peels. While some studies have investigated *Citrus limon* essential oils in Morocco in general[32,33], no research has yet focused on the Pre-Rif region. This gap highlights the originality and novelty of the present study, which aims to provide the first insights into the chemical composition and potential applications of *Citrus limon* peel essential oil from this underexplored area.

To our knowledge, this is the first study to characterize the essential oil composition and antimicrobial properties of *C. limon* peels specifically from the Moroccan Pre-Rif region, an area with unique pedoclimatic conditions that may yield distinct phytochemical profiles. This originality strengthens the scientific and practical relevance of our work. In this framework, the present study sought to (i) determine the chemical composition of *C. limon* peel essential oil (EO) from Morocco's Pre-Rif region through gas chromatography–mass spectrometry (GC–MS), (ii) evaluate its antibacterial and antifungal properties against clinically and agriculturally relevant microorganisms, and (iii) explore its potential contribution to sustainable waste valorization, environmental preservation, and the development of natural antimicrobial agents for applications in health, food safety, and eco-friendly crop protection.

2. Materials and Methods

2.1. Collection and Preparation of Citrus Peels

A total of approximately 130 lemon fruits were harvested from three representative orchards in the Pre-Rif region (Taza, Morocco) in March 2022. In each orchard, 5 to 10 trees were randomly selected, and for each tree, 3 to 5 mature and healthy fruits were collected. The plant material used in this research was *Citrus limon* (lemon), taxonomically validated by Professor Zidane, botanist at the Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco. After harvest, the fruits were rinsed with tap water, dried on sterile paper towels, and peeled by hand. The peels were cut to approximately 2–3 cm pieces and placed either directly into essential oil (EO) extraction or kept at −30 °C and processed within 24 h.

2.2. Extraction of Essential Oil

EOs were obtained from hydrodistillation using a Clevenger-type apparatus. Approximately 400 g of fresh lemon peels were placed in a flask with distilled water (5 L). After boiling had started, the distillation was carried out for 3 h. The oil was collected, dried with anhydrous sodium sulfate, and stored in amber vials at 5 °C until used. The extraction yield (%) was determined by:

$$Rdt(\%) = \frac{V_{HE}}{m_{ms}} \times 100$$

With: Rdt (%): Yield of essential oils (mL/g)

V_{HE}: Volume collected of essential oil

m_{ms}: Mass of lemon peels

2.3. Chemical Composition Analysis (GC–MS)

The composition of essential oil (EO) was analyzed by gas chromatography–mass spectrometry (GC–MS) using a Thermo Trace 1300 gas chromatograph connected to a TSQ 8000 EVO triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The GC–MS system contained a TR-35MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The following analytical parameters were used: helium as carrier gas (1.5 mL/min), injector temperature: 200 °C, injection volume: 1 µL, split mode 1:33. The heating program started at 40 °C for 2 min, ramped to 180 °C at 4 °C/min, then ramped to 300 °C at 20 °C/min for 2 min. The transfer line was maintained at 250 °C.

To ensure quality control (QC) and quality assurance (QA), all analyses were performed in triplicate. Calibration was conducted using a homologous series of n-alkanes

(C8–C20). Compound identification was validated by comparing both retention indices and mass spectral data against the NIST and Wiley libraries. Instrument performance was verified daily using reference standards to maintain analytical accuracy and reproducibility.

2.4. Antimicrobial Activity

The antimicrobial activity of the EO was assessed using the broth microdilution method in sterile 96-well microplates. Mueller–Hinton broth was employed for bacterial strains, while Sabouraud broth was used for fungal cultures. A stock solution of EO (100 mg/mL) was prepared in 0.2% agar and serially diluted (1/2 to 1/64). Each well was filled with 100 µL of broth followed by 100 µL of the corresponding EO dilution. Subsequently, 10 µL of microbial suspension was added (10^6 CFU/mL for bacteria and 10^4 CFU/mL for fungi). The plates were incubated for 24–48 h at 37 °C for bacteria and at 26 °C for fungi. After incubation, 10 µL of resazurin solution (5 mg/mL) was added to each well, followed by a further 3 h incubation. A color change from purple to pink indicated microbial growth. The Minimum Inhibitory Concentration (MIC) was defined as the lowest EO concentration at which no color change occurred.

To determine Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC), 10 µL from wells exhibiting no visible growth were plated on Mueller–Hinton agar (bacteria) and Sabouraud agar (fungi), and incubated at 37 °C (bacteria) and 26 °C (fungi). MBC/MFC was the lowest concentration of an EO that reduced viable counts by ≥ 99.99%. The MBC/MIC or MFC/MIC ratio was then used to classify activity as being bactericidal/fungicidal (< 4) or bacteriostatic/fungistatic (> 4).

The test panel included clinically relevant Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*), Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), and the opportunistic fungus *Candida albicans*. The strains of microbes used were from the microbial holdings of the National Institute of Aromatic and Medicinal Plants (Morocco) and are recognized for their roles in nosocomial infections and their associated patterns of antimicrobial resistance. In particular, *P. aeruginosa* and *K. pneumoniae* are known to exhibit some of the highest levels of intrinsic resistance; *S. aureus* (including MRSA strains) poses significant clinical risks; and *C. albicans* is regarded as an important agent of fungal infections for patients at compromised immunological states.

All antimicrobial assays were performed in three independent experiments (biological replicates, conducted on different days). For agar diffusion tests, each condition (strain × concentration) was tested with three discs per plate (technical replicates), yielding n = 9 measurements per condition and strain. For MIC/MBC (broth microdilution), each independent experiment was run in duplicate wells, giving n = 6 observations per condition.

A positive control antibiotic was included for bacteria (gentamicin, 10 µg/disc, for diffusion; gentamicin 0.5–8 µg/mL for MIC) and a positive control antifungal for yeast/mold (amphotericin B, 0.25–4 µg/mL). A negative (vehicle) control matched the highest solvent fraction used for EO stocks (≤ 1% DMSO and 0.1% Tween-80 in sterile saline), and a blank without antimicrobial was included to check sterility. Inocula were standardized to 0.5 McFarland and adjusted to ~1×10^6 CFU/mL for diffusion and 5×10^5 CFU/mL for broth microdilution. Incubation was at 37 °C for 18–24 h (bacteria) and 28–30 °C for 24–48 h (yeast/mold).

No human or vertebrate animal subjects were involved. All work with microorganisms was conducted under BSL-2 biosafety practices in the institutional microbiology facility. No environmental release occurred, and all biological waste was autoclaved prior to disposal.

2.5. Statistical Analysis

Results are reported as mean ± standard deviation (SD). Data normality was assessed using the Shapiro–Wilk test. When assumptions were met, one-way ANOVA followed by Tukey's HSD test was applied. The significance threshold was set at $p < 0.05$.

3. Results

3.1. Extraction Yield

Hydrodistillation of *Citrus limon* peels collected in the Pre-Rif region of Morocco yielded 0.5% (v/w) essential oil, based on fresh peel weight, equivalent to 0.5 mL per 100 g of raw material. The obtained oil exhibited a pale yellow color and a characteristic lemon scent, and the quantity recovered was sufficient for subsequent chemical and biological analyses. This yield reflects the efficiency of the hydrodistillation process under the applied conditions (Clevenger-type apparatus, 3 h distillation, peel fragments of 2–3 cm). The essential oil was stored in amber vials at 5 °C to preserve its volatile constituents prior to GC–MS analysis.

3.2. Chemical Composition

The chromatographic analysis of *Citrus limon* essential oil (**Figure 1, Table 1**) indicates that the chemical constituents of the oil are largely D-limonene (46.89%), the cyclic monoterpene related to citrus fruits, from which it derives its strong aroma and well-established biological features including antioxidant, anti-inflammatory, and antimicrobial properties. The other monoterpenes also enhance the anti-infectious and anti-inflammatory properties, with α-pinene present at 6.81% and α-myrcene at 1.21% (**Figure 2**). The addition of oxygenated components such as citral (8.32%), an aldehyde, with strong antibacterial properties, linalool (2.41%), geraniol (1.70%), and nerolidol (1.09%) extends the oil's medicinal scope, especially for the oil's calming, sedative, and antifungal abilities. Unusual components include ethoxymethylbenzene (7.86%) and brominated cyclopentane (6.39%).

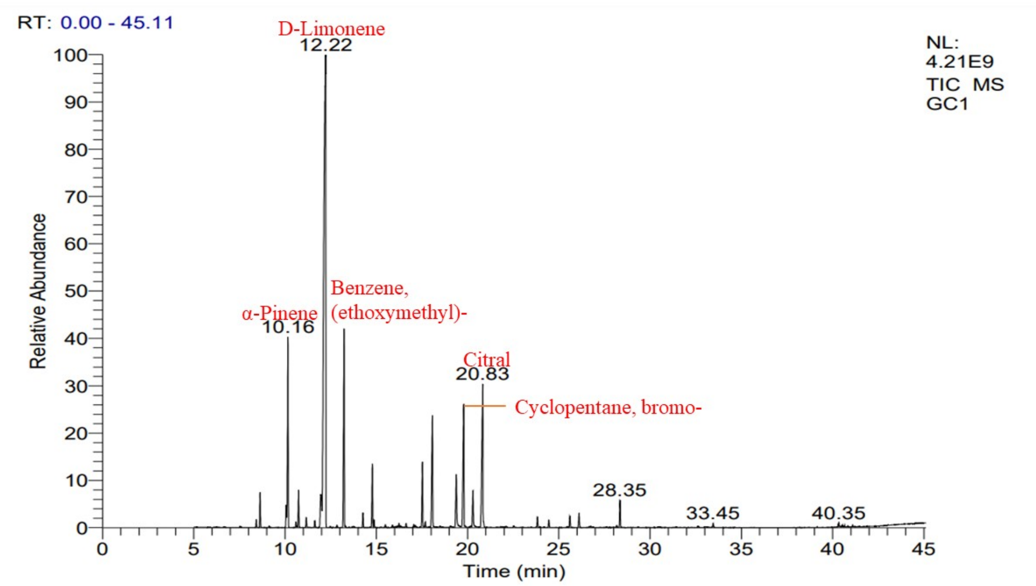

Figure 1. Gas chromatography–mass spectrometry (GC–MS) chromatogram of *Citrus limon* peel essential oil (EO) extracted from fruits cultivated in the Pre-Rif region, Morocco. Peaks correspond to the major volatile compounds, with D-limonene (46.89%) being the most abundant, followed by citral, benzene (ethoxymethyl)-, α-pinene, and cyclopentane bromo-.

Table 1. Chemical composition of *Citrus limon* essential oil.

Number	Retention Temps	Compounds	Content (%)
1	8.64	Tricyclo [2.2.1.0(2,6)] heptane, 1,3,3-trimethyl	1.10
2	10.07	Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene-, (1S)-	0.81
3	10.16	α-Pinene	6.81
4	10.74	α-Myrcene	1.21
5	11.16	Octanal	0.35
6	11.97	Benzene, 1-ethyl-2,4-dimethyl-	2.40
7	12.22	D-Limonene	46.89
8	13.23	Benzene, (ethoxymethyl)-	7.86
9	14.27	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	0.53
10	14.78	Linalool	2.41
11	17.53	Eucalyptol	2.55
12	18.07	1,2-Bis (3-cyclohexenyl) ethylene	5.33
13	19.38	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	2.79
14	19.79	Cyclopentane, bromo-	6.39
15	20.29	Geraniol	1.70
16	20.83	Citral	8.32
17	23.83	Bicyclo [2.2.1] heptane, 7,7-dimethyl-2-methylene	0.43

Table 1. *Cont.*

Number	Retention Temps	Compounds	Content (%)
18	25.61	Hexane, 1-chloro-5-methyl-	0.46
19	26.10	Bicyclo [3.1.1] hept-2-ene-2-ethano l, 6,6-dimethyl	0.57
20	28.35	Nerolidol	1.09

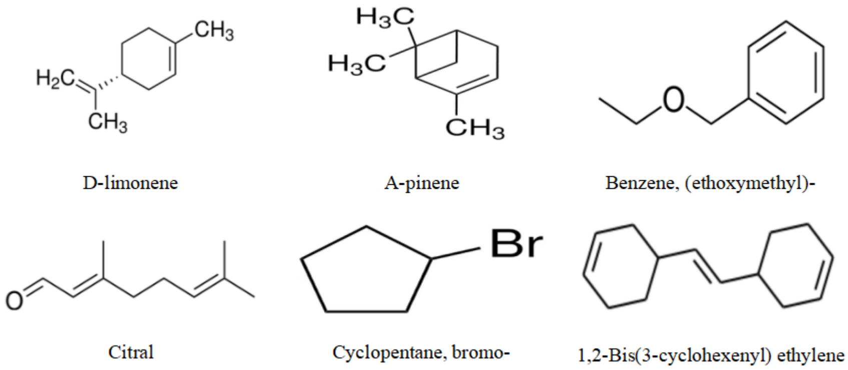

Figure 2. Chemical structures of the principal constituents identified in *Citrus limon* essential oil: D-limonene, α-pinene, benzene (ethoxymethyl)-, citral, cyclopentane bromo-, and 1,2-bis(3-cyclohexenyl) ethylene. These compounds are responsible for the antimicrobial and antioxidant activities of the oil.

3.3. Antimicrobial Activity

The table below presents the antimicrobial activity of Citrus limon essential oil (EO) against six clinically relevant microorganisms. The recorded Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal/Fungicidal Concentration (MBC/MCF) values indicate that C. limon EO exhibits broad-spectrum antimicrobial potential, demonstrating both bactericidal and fungicidal effects depending on the target organism (**Table 2**).

Table 2. The antimicrobial activity of *C. limon* essential oil.

Species	MCI (mg/mL)	MCB or MCF (mg/mL)	Ratio (MCI/ MCB)	Effect
Escherichia coli	13.3 ± 0.6^{a}	**26.5 ± 1.2**^{a}	0.5 ± 0.03^{b}	Bactericide
Staphylococcus aureus	**6.6 ± 0.4**^{b}	**6.6 ± 0.4**^{c}	1.0 ± 0.05^{a}	Bactericide
Pseudomonas aeruginosa	**6.6 ± 0.4**^{b}	**6.6 ± 0.4**^{c}	1.0 ± 0.05^{a}	Bactericide
Klebsiella pneumoniae	13.3 ± 0.6^{a}	**26.5 ± 1.2**^{a}	0.5 ± 0.03^{b}	Bactericide
Bacillus subtilis	13.3 ± 0.6^{a}	13.3 ± 0.6^{b}	1.0 ± 0.05^{a}	Bactericide
Candida albicans	**1.7 ± 0.1**^{c}	**1.7 ± 0.1**^{d}	1.0 ± 0.05^{a}	Fungicide

Values followed by different letters within each column are significantly different according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

Of the evaluated bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus* had the lowest values of MIC (6.63 mg/mL) and the same MBC values (6.63 mg/mL), producing an MIC/MBC ratio of one suggesting a bactericidal action at the inhibitory doses. *Bacillus subtilis* had the same MIC and MBC (13.25 mg/mL), demonstrated with an MIC/MBC ratio of one producing lethality at the lowest inhibitory concentration. The fungal strain *Candida albicans* represented the most sensitive organism, with an MIC and MCF of only 1.66 mg/mL, again showing the fungicidal potential of the essential oil most likely due to other constituents (e.g. citral and linalool) that have been shown to disrupt fungal membranes. In contrast, the Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* had lower susceptibility as both bacteria have complex outer membranes, presenting an MIC of 13.25 mg/mL and MBC of 26.50 mg/mL (MIC/MBC ratio = 0.5). Although the higher dose needed for complete killing, there was still a bactericidal impact. These results demonstrate promise for C. limon essential oil as a potential antibacterial, specifically with multidrug resistant organisms such as *P. aeruginosa* and *C. albicans*. The lower MIC/MCF values and the consistent bactericidal or fungicidal ratios suggest that *C. limon* EO may be a potential natural antibacterial agent alone or in combination with traditional medications. Further *in vivo* studies and mechanistic studies should be conducted to further verify its potential use in medicine and industry.

4. Discussion

Although numerous studies have reported on the chemical composition and antimicrobial activities of citrus essential oils worldwide, little attention has been given to Moroccan ecotypes, and no research has specifically investigated the Pre-Rif region. The unique pedoclimatic conditions of this area are likely to influence the phytochemical profile of *C. limon* peels, which has not been critically addressed in previous literature. By filling this gap, the present study not only provides new insights into the variability of citrus essential oils but also contributes to the development of sustainable waste valorization strategies tailored to local agricultural contexts. A schematic graphical abstract summarizing the main findings of the present study is provided in **Appendix A** (**Figure A1**).

4.1. Extraction Yield

The essential oil yield obtained in this study (0.5%) is in line with values that have been previously reported, but it can vary substantially based on the citrus species, its geographic origin, and extraction protocol. For example, previous hydrodistillation studies of citrus peel listed yields of 0.05% to 0.38%, respectively [34]. Pakistan reported that yields of essential oils from fresh peels of *C. reticulata* 0.30%, *C. paradisii* 0.20%, and *C. sinensis* 0.24%, respectively [35]. Another report also found *C. reticulata* peels to yield 0.29% [36]. In Iran, the yield of *C. aurantium* essential oil reached 0.7% [37]. More recently, reports from Morocco found higher yields of essential oil from *C. limonum* extract were found with a 1.02% yield, *C. reticulata* 0.80% yield, and *C. paradisii* 0.90% yield [33].

An important parameter that may have contributed to differences in yield between studies is the plant material: both the present study and those from Pakistan used whole peel fragments to extract their essential oils while the Moroccan study used only the zest of the peel which, in general, contain higher levels of essential oil. Further, differences in extraction method, maturity of fruit, post-harvesting treatments, and soil type, environment and climate (e.g., altitude) may have a large effect on yield of essential oil [38,39].

4.2. Chemical Composition

Research conducted by Pu et al. [40] reported that the chemical profile of *C. limon* was predominantly composed of α-fenchene (30.19%), D-limonene (21.74%), and linalyl formate (15.46%). In contrast, the study by Bouzenna et al. [41] reported a chemical profile dominated by bioactive constituents such as Z-citral (53.21%), neryl acetate (13.06%), geranyl acetate (10.33%), and limonene (4.23%). Interestingly, several reports have documented limonene levels reaching up to 60% in essential oil [42]. Additionally, the minimal concentrations of citral, neryl acetate, and geranyl acetate have historically been high in EO [42,43].

Overall, the chemical composition of *C. limon* essential oil (EO) shows a high degree of similarity across different studies, with limonene consistently reported as the dominant constituent, followed by varying proportions of β-pinene, γ-terpinene, citral, and oxygenated monoterpenes such as α-terpineol and terpinen-4-ol. Comparative analyses revealed some regional differences: Indian samples were characterized by limonene levels ranging from 29.0% to 53.6%, alongside notable amounts of β-pinene, γ-terpinene, citral, and α-terpineol; Turkish samples displayed higher limonene content (up to 78.9%) with lower proportions of other monoterpenes; and Greek samples contained 59.3% limonene, 13.4% β-pinene, 8.6% γ-terpinene, and smaller amounts of β-myrcene and geranial. These findings indicate that while limonene remains the principal component of *C. limon* EO, the relative abundance of accompanying compounds can vary according to geographical origin and extraction conditions [44–47].

The differences in chemical composition between studies reporting on essential oils of *Citrus limon* could be described as two major factors: the location of the plant source and the methods used in the studies to identify and quantitate the components of the oils. The geographical factors are important in the synthesis of secondary metabolites, like the components of essential oils. The environmental differences, as related to geography, including soil characteristics, temperature, altitude, water availability, and light exposure, have a significant impact on plant metabolic pathways and result in differences in quantity and quality of volatile components. A limon tree grown in a coastal Mediterranean environment will have its own profile of monoterpenes and monoterpenoids compared to a similar tree grown in a subtropical environment, or at high altitude, etc. The agricultural practices used for the tree may also impact the chemical profile of the limon tree, these practices can include using organic fertilisers or irrigation methods.

The methods for identifying and quantifying chemical constituents can create inconsistencies across studies. Methods vary in sensitivity, chemical library databases, and resolution. The type of GC column or temperature program, carrier gas, and detection limits are all important factors which may affect the identification and quantification of the chemicals. With respect to precision some studies may rely solely on retention indices and library matches, while others validate compounds using authentic standards, increasing accuracy. As a result, it is important to consider agro-environmental factors and analytical methods when looking at the chemical profiles of *Citrus limon* essential oils in studies, as both affect the final compositional fingerprint. All factors need to be properly documented and standardised, especially for comparative or industrial uses, to ensure repeatability, reliability, and correct interpretation of the activity or quality of the essential oil.

4.3. Antimicrobial Activity

The present findings indicate that *C. limon* essential oil exhibited the strongest antimicrobial activity, with lower Minimum Inhibitory Concentration (MIC) values compared to other citrus samples previously reported [34]. The observed differences in antimicrobial efficacy can be attributed to the distinct chemical composition of these essential oils. While limonene is the predominant component in most other citrus cultivars, *C. limon* EO contains a complex mixture of compounds–including limonene, β-pinene, α-pinene, p-cymene, and various minor constituents–that likely act synergistically, enhancing its overall effectiveness. Supporting this, previous studies in Morocco reported MIC values of 60 µg/mL for *S. aureus* and 750 µg/mL for *E. coli* for *C. limon* essential oil [33]. A study conducted in Egypt also reported the MIC values of *C. limon* essential oil against Bacillus cereus (510 µg/mL), *Escherichia coli* (260 µg/mL), *Pseudomonas aeruginosa* (200 µg/mL), and *Staphylococcus aureus* (430 µg/mL) using the microdilution method [48]. The effects of *C. limon* essential oil observed in this study differ from those reported in previous research. Also, the bioactivity of the *C. limon* essential oils, as indicated in multiple studies, is also different from that of other species. These differences can be accounted for by the different chemical compositions of the essential oils and the diversity of bacterial strains tested in this study. In a solution, the presence of phenols and alcohols can directly affect bactericidal and bacteriostatic activity. The antimicrobial action of bioactive compounds is generally classified in the following order: Phenols (very active) > Alcohols > Aldehydes > Ketones > Ethers > Hydrocarbons [49]. Beyond its biological properties, the antimicrobial potential of *C. limon* essential oil also highlights its value in broader sustainability and socio-economic contexts. The following sections discuss how transforming citrus peel waste into essential oils aligns with circular economy principles and supports rural development.

4.4. Environmental and Sustainability Perspectives

Transforming citrus peel waste into essential oils represents a multi-dimensional contribution to environmental protection, sustainable development and social and economic benefits [50]. Environmentally, the substitution of natural citrus essential oils (CEOs) for synthetic pesticides, preservatives, and antibiotics greatly reduces the consequences of toxic residues in the soil, water and food chains. This improves not only soil quality and water quality but also biodiversity in agroecosystems. The natural antimicrobial, antioxidant and insecticidal properties of the CEOs provide alternatives to agrochemicals which also help reduce the risks of pesticide resistance, and also reduce one of the greenhouse gas emissions that is created because of synthetic chemistry. These properties fit nicely with green chemistry and can help reduce the ecological footprint associated with agricultural practices and food preservation.

Citrus peel valorization using citrus peel transformation takes large amounts of agro-industrial waste and turns it into high-value products. Each year there is over 60 million tons of citrus agro-industrial waste produced around the world; the waste that is most important for citriculture is the citrus peels. Citrus peels provide by-products to provide essential oils, flavonoids, or other bioactive compounds which could eventually valorize organic wastes, decrease use of landfilling space from food waste, and decrease methane emissions from landfilling. If citrus by-products could be utilized/heavily valorized in the value chain of industrial, pharmaceutical, and cosmetic applications then a systems oriented closed-loop with continuous resource looping, significant waste reductions, and waste-to-value creation could occur. Moreover, not only would extracting bioactives to valorizing products reduce waste, but this work would give local producers and farmers that work as a farmer cooperative an opportunity, to extract these bio-resources as the industry would give local producers the ability to extract the bio-resources allowing the farmer to generate an appropriate form of retribution that allows the farmer to strengthen their socio-economic resilience in the rural community [51].

CEOs provide safe, naturally sourced substitutes that minimize the population's exposure to chemicals from a health and wellness perspective [52]. The biological-pharmacological properties of these compounds are established, and they contain antimicrobial, anticancer, anti-inflammatory, and antiaging activities that can be used within medicine, food preservation, and cosmetics. (Using fewer synthetic drugs and preservatives builds a more sustainable and holistic approach to health care) [53]. For example, *Citrus limon* has gastroprotective activity and antitumor activity; and *C. bergamia* and *C. sinensis* are linked to stress reduction and improved sleep quality. Citrus bioactivities expand the conversation about citrus waste valorisation beyond environmental sustainability actions to actions for public health. In reality, they align with the UN's Sustainable Development Goals (SDGs), especially SDG 12 (Responsible consumption and production) related to circular valorisation of agro-waste, SDG 3 (Good health and well-being) related to natural and safe bioactive compounds, and SDG 13 (Climate Action) involving reduced pollution from food-derived waste and less reliance on fossil-fuel based agrochemicals [54]. Thus, citrus peel valorisation constructs not only the effectiveness of waste management, but also an integrative paradigm for environmental sustainability, economic innovation, and health for society.

4.5. Potential of *Citrus Limon* Essential Oil in Sustainable Crop Protection

The use of *Citrus limon* essential oil (EO) presents a sustainable and environmentally-friendly alternative to traditional pesticides in the context of Integrated Pest Management (IPM) [55]. While its antifungal activity has been adequately documented against phytopathogens such as *Botrytis cinerea* and *Fusarium oxysporum* (which result in significant losses both pre- and post-harvest worldwide), these antifungal effects are primarily mediated by the presence of monoterpenes, primarily limonene, γ-terpinene, and β-pinene among others, that disrupt the fungal membrane, intracellular enzymatic activity and germination of spores. Besides antifungal activity, lemon EO is entomopathogenic and acaricidal against key horticultural pests such as *Aphis* spp., *Bemisia tabaci*, and *Spodoptera littoralis*, with its bioactivity showing neurotoxic effects (physiology) against pests, disruption of respiratory metabolism, and damaging cuticle integrity, contributing to decreases in either survival or reproductive success. Additionally, EO-based products are biodegradable, transferring to a different environmental chemistry with no persistence or accumulation relative to many of the synthetic pesticide products. Consequently, the rapid degradation of EO-based pest products has implications for the use of these products in agriculture well as for certification schemes used for organic agriculture.

It has been reported that EO-based biopesticides are generally less hazardous to non-target organisms compared to synthetic pesticides [56]. Some studies have noted decreased predation, parasitization and mobility of beneficial insects (e.g., pollinators) due to EO exposures. This points to the need to conduct additional ecotoxicological research. As for terrestrial invertebrates, they may be less fragile; however, longer field studies are needed to indicate safe limits for EO application. From a sustainability perspective, *C. limon* essential oil (EO) applied within ex-filtration of processing residues valorizes by-products from citrus processing. Citrus peel residues that constitute organic waste are converted into high-value bioproducts. In turn, there is reduced environmental pollution from synthetic pesticides and alternative sources of revenue for local farmers and cooperative help to address rural development and circular bioeconomy [53].

Across the world, the growing application of green crop protection is increasingly favouring biopesticides derived from plants. Essential oils are now being classified as multifunctional products (i.e., antifungal, insecticidal, ovicidal, and larvicidal), and it is well-known that they also have many modes of actions [57]. The multiple modes of actions associated with essential oils reduce the chances of resistance, and resistance development is a major limitation of conventional pesticides. Moving forward, innovative technologies, such as nanoencapsulation methods and agrinanotechnology may provide solutions to improving EO stability, protect from volatility, and slow release to improve field efficacy while minimizing exposure to non-target organisms [58]. These innovations can provide the means to take EOs from laboratory efficacy to field application. In summary, deploy of *C. limon* essential oil (EO) with IPM directly supports goals of sustainable agriculture and food security while remaining relevant to the United Nations Sustainable Development Goals (SDGs), specifically SDG 12 (Responsible Consumption and Production), SDG 3 (Good Health and Well-Being), and SDG 13 (Climate Action). *C. limon* essential oil (EO) integrates ecological safety, valorization of agri-waste, and is compatible with organic agriculture, making it a foundational element in crop protection in the future.

4.6. Advances in Formulation and Delivery Systems

Though essential oils (EOs) have many types of antimicrobial, antioxidant, and pesticide activity, their use in agriculture and industry is often limited by their volatile structure, low soluble content, and the potential for chemical instability. Due to the structure and chemical nature of essential oils, they are often evaporated, or degrade under environmental stressors (e.g. light, oxygen, temperature), and lose their persistency on treated surfaces, resulting in decreased efficacy in the field. In recent years, researchers have attempted to overcome these limitations by developing new formulation and delivery systems to improve the stability, bioavailability, and efficacy of EOs. Emerging as one of the most promising avenues of research is nanoemulsion technology. By delivering antiviral, antifungal, bacterial, or pesticidal properties as a nanoemulsion and, in essence, bringing droplet sizes down to the nanometre scale, there are increased estimations of surface area for interaction with either microbial cells or the cuticle of pests, thereby improving antimicrobial efficacy over conventional applications. In addition, nanoemulsions promote the dispersion of hydrophobic EOs into aqueous environments (i.e. sprays used to applied in agriculture) by improving solubility of the EOs and ensuring the application remains uniformly applied to the surface. The small droplet sizes also promote penetration through plant tissue, and microbial cell membranes, improving bioavailability of the desired effect. For every decrease in EOs products is also a decrease in potential environmental impact and monetary costs to apply these EOs. Recent studies have shown that pest and pathogen control was achieved at lower rates of application of EO-based nanoemulsions in comparison to other formulations.

Another method is microencapsulation, where your EO droplets are enveloped in a protective wall material, typically polymers, starch, or proteins. Microencapsulation allows controlled release and restrictions on the degradation of active ingredients from light, oxygen, or temperature instability. Microencapsulation also conceals the pungent OE aroma, makes the product more user-friendly, increases the EO-formulation's shelf-life, and also, encapsulated EOs will have a longer bioactivity potential, an especially desired trait you want to maintain for consistency in pest and pathogen suppression in the field. Biodegradable polymer coatings offer more sustainable delivery of EOs with stability and durability. Bioactive coatings protect essential oils from environmental pressures or stressors, while allowing for slow and sustained delivery of bioactive ingredients. Formulations with biodegradable polymers or biodegradable materials that include EOs produce a minimized environmental risk and complement the concept of sustainable agriculture. Polymer-coated EOs can be used for crop protection to seeds, fruit, or leaves and offer long-lasting protection with little to no repeated applications.

In addition to this, the combination of novel formulation technologies with 'green' nanotechnology and clever delivery systems presents exciting potential. For example, stimuli-responsive carriers that release vital compounds or secondary metabolites based on environmental stimuli (humidity, alterations in the pH, attacks by pests etc.) could potentially increase dosing accuracy of EO-based biopesticides and work to improve efficacy while reducing unwanted non-target effects and which may also reduce the impact of the EO-based product on non-target users such as beneficial insects, aquatic organisms, and soil microbiota. Indeed, formulations and delivery systems create an innovative opportunity to move past the inherent limitations of essential oils, making them worthy of consideration in the markets for sustainable agriculture. When the technology involves the use of formulations that have the potential to enhance stability and persistence while having specific action responses (e.g., nanoemulsions, microencapsulation, and/or the use of biodegradable coatings, etc.) or being functional as an eco-friendly alternative to synthesized agrochemicals and thus becoming a part of the transition to sustainable and resilient crop protection products for farmers.

4.7. Socio-Economic and Rural Development Implications

Building on the demonstrated antimicrobial efficacy, the valorization of citrus peel residues also carries socio-economic implications, particularly for citrus-producing regions such as the Moroccan Pre-Rif. These implications connect the scientific findings to practical applications and local development. In addition to its environmental and agronomic benefits, the valorisation of citrus peel waste into essential oils has substantial socio-economic benefits for citrus-producing countries like Morocco. Citrus production is an important agricultural activity in the Pre-Rif Region, as well as other areas of Morocco, although the tons of by-products that are produced create an environmental burden and, unfortunately, little additional revenue. The recovery of these residues into essential oils represents an exciting new opportunity that creates a nexus between sustainability and economic empowerment. For small-holder producers and rural cooperatives, the valorisation of citrus peel presents an entirely new income diversification opportunity. Rather than regarding their peels as waste, they can now be collected, processed, then concentrated and added value through refining into high-value essential oils, and with little or no costs at all to manage disposal and disposal costs and generate another profit center. This presents an opportunity for local processing, in local small-scale facilities and local cooperatives; this offers benefits to community based entrepreneurship and long term benefits to employment within the surrounding villages, particularly women and youth.

More specifically, established local EO-based industries mean rural development or communities achieving an economic independence. Farming communities having a different alternative use for agricultural waste by-products decreases their economic risk exposure. new circular economy prospects may initiate knowledge transfer and technology innovations to rural communities to improve smallholder farmers' engagement and opportunities along sustainable agriculture value chains. Development in local EO production supports national agricultural resilience and food sovereignty with a diminished reliance on imported synthetics pesticides and preservatives. This circular local nature of the natural, locally sourced biopesticides also supports organic and low input farming systems. This is in compliance with Morocco's agricultural directions and international obligations to sustainability. The production of high-quality EO raw products (bioproducts) from peel waste of Moroccan citrus enhances Morocco's export potential; this will strengthen Morocco's role in the rapidly growing global marketplace for natural antimicrobials, biopesticides, and green cosmetics. Valorizing the peel of citrus is achieving an environmental strategy and stimulating rural development to create employment and sustainably establish bio-based industries in landscapes and cultural ecosystems at a scale of green eco-value.The citrus valorization angle and potential for added value in rural development allows bridging communities, industries and sustainability to create something a win-win scenario across the families of farmers, their rural communities, and the agrarian economies in which they work.

4.8. Limitations

Although this study sheds light on the chemical composition and antimicrobial properties of essential oil derived from *C. limon* from the Moroccan Pre-Rif region, it is worth acknowledging several limitations. The present findings are derived from laboratory-based *in vitro* assays, and no field trials were conducted to establish the efficacy the essential oil in natural agricultural environments. Furthermore, long-term efficacy and stability studies, as well as formats (e.g., emulsified preparation or water-soluble formulation), were all omitted and this may impact their potential applications to crop protection and/or food preservation. Future research should give consideration to these factors if it is hoped to enhance the wider applicability and scalability of this potentially valuable natural product.

4.9. Future Research Directions and Perspectives

The positive results of this work reveal the need to further research from laboratory success to practice. There are multiple research routes to follow to fully harness *Citrus limon* essential oil (EO) in agricultural and health habits successfully and sustainably. Although the antimicrobial activity of C. limon EO has been shown in vitro, its efficacy under realistic agronomic conditions has not been broadly tested. Greenhouse and field experiments must be conducted to verify its bioactivity against key crop pathogens and pests, while controlling for environmental conditions like temperature, humidity, UV exposure, and plant physiology. Through such experiments, we will understand the longevity of EO functionality, its effective spectrum of phytosanitary efficacy, and its compatibility with existing Integrated Pest Management (IPM) approaches.

One of the primary issues with essential oils is their volatility, hydrophobicity and sensitivity to degradation. Future studies should focus on using sophisticated formulations methods like nanoemulsions, encapsulation or biodegradable coatings to enhance stability, solubility and controlled release. Moreover, C. limon EO may have more synergistic effects with other natural bioactive compounds, allowing for a possible increase in microbial and pest inhibition as well as reducing the dose required. Optimized formulations will be beneficial in providing cost-effective and scalable EO-based products. Prior to applying C. limon EO on the market, it is important to assess the possible impacts from the C. limon EO on human health, beneficial insects (such as pollinators), soil microbes, and aquatic systems. Findings from toxicological and ecotoxicological studies clarify why it is necessary to conduct these toxicological and ecotoxicological studies will determine impact thresholds for safe applications, degree of acceptability, exempt or dangerous wastewater components and complete toxicity rates predictions. This can apply to a wide range of regulations, consumer safety demands, and confirms sustainability from an ecological perspective as well as acceptance by organic farming systems.

The bioactive properties of *Citrus limon* peel essential oil (EO), combined with its antimicrobial efficacy, suggest promising applications beyond conventional formulations. Encapsulation of the EO into nanoemulsions or other nanoformulations could enhance its stability, controlled release, and target specificity, thereby improving efficiency and reducing the required doses. Moreover, integrating EO-based biopesticides into integrated pest management (IPM) systems offers a sustainable approach to crop protection, minimizing reliance on synthetic chemicals while preserving environmental and human health. These strategies could significantly broaden the practical and economic impact of utilizing Moroccan citrus waste.

Despite its potential bioactivity, large-scale use of *C. limon* EO will ultimately depend upon its economic viability. Future work will need to determine the extraction efficiency at commercial scale, the cost-benefit ratio for ultimate consumers, and the sustainability of production chains as a whole. Importantly, the valorization of local residues of citrus processing will also be a priority, with a goal of minimizing novel waste disposal costs while adding value to rural communities. With these studies, we can determine whether benefits of large-scale EO production can compete with or supplement existing synthetic agrochemicals in the marketplace. Ultimately, the successful commercialization of *C. limon* EO for agricultural and healthcare use will also require transparent regulatory pathways. There will be a need for policies that adequately address required safety limits, quality control standards, and international regulatory frameworks governing biopesticide and natural preservative production. Collaboration with policymakers, regulatory agencies and farmer cooperatives will be critical to establish EO-based strategies as legitimate weaponry within integrated systems of crop protection and public health.

5. Conclusions

This study characterized the chemical composition and antimicrobial properties of *Citrus limon* peel essential oil from Morocco's Pre-Rif region. *C. limon* peel essential oil is rich in bioactive metabolites such as D-limonene, citral, and α-pinene, and exhibited strong inhibitory activity against pathogenic bacteria and fungi, thereby confirming its potential to constitute a naturally antimicrobial agent for health and agricultural applications. By integrating the chemical characterization of *Citrus limon* peel essential oil, its demonstrated antimicrobial efficacy, and sustainability considerations, this study highlights the innovative potential of valorizing Moroccan citrus waste. This approach not only advances scientific understanding of local citrus ecotypes but also proposes practical strategies for transforming agricultural by-products into high-value bioproducts, thereby linking research novelty with socio-economic and environmental benefits. Indeed, the research not only typified the biological landscape, but also demonstrated the importance of valorizing citrus waste as a pathway to sustainable crop protection, and especially with citrus peel by converting discarded residues of fruit waste into high-value bioproducts, therefore taking the approach of cascading and revitalizing, whilst considering innovations linked to circular economy models, environmental pollution reduction, and value into the environment and actionable eco-friendliness when considering all pesticides, and the engaging shifting landscape to bio-based alternatives. Somewhere in this journey the responsibility for meeting critical environmental challenges, stimulating local rural development and bio-based industry emergence are clear components of benefits associated with valorization pathways. Overall, the *C. limon* peel essential oil entailed a meaningful pathway toward sustainable agriculture and sustainable crop protection associated with phytochemical innovation and social and economic benefit. Further understandings will be discovered. Further understandings will be discovered through further acceptable formulation technologies, field-assembled and identifiable regulatory approvals, to follow through with bioproduct-based sustainable strategies into Integrated Pest Management systems as a green alternative.

Author Contributions

Conceptualization, H.K. and Y.M.; methodology, O.C.; validation, O.C., A.S. and H.E.F.; formal analysis, H.K. and Y.M.; investigation, H.K., S.E.A. and M.T.; resources, A.L.; data curation, H.K. and A.S.; writing–original draft preparation, H.K. and Y.M.; writing–review and editing, N.M., A.L. and E.M.A.; supervision, H.E.F., A.L. and E.M.A.; project administration, E.M.A.; visualization, Y.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The datasets generated during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

Appendix A

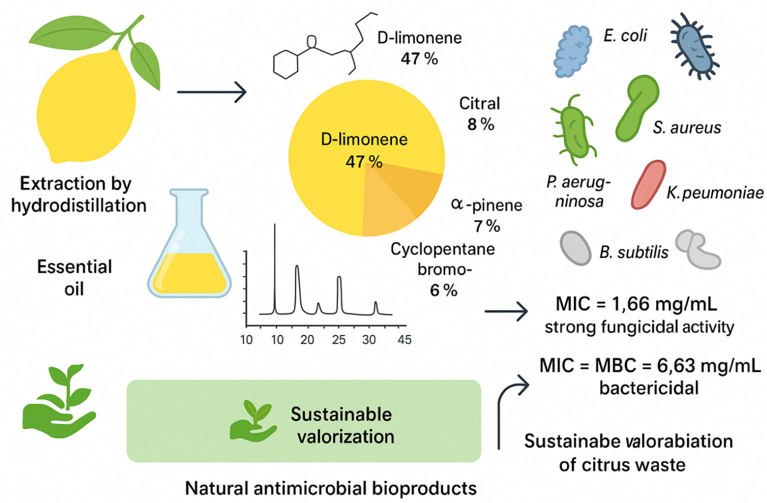

Figure A1. Graphical abstract illustrating the main findings of the study.

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