



Research Article



Evaluation of Antibacterial Activity of Essential Oil Extracted from the Peels of *Citrus limetta* Against the Enteric Fever Pathogen *Salmonella enterica* serovar Typhi

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Abstract:

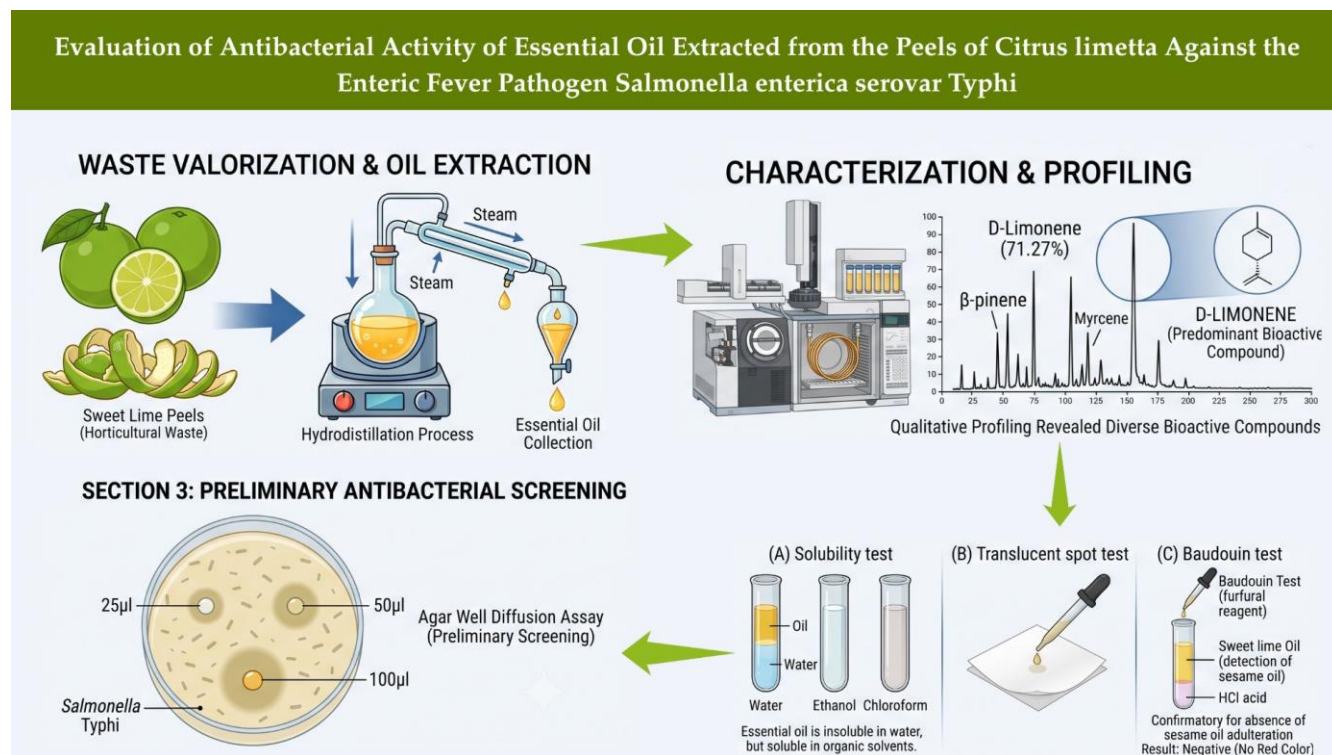
Horticultural waste, though historically overlooked, serves as a valuable reservoir of bioactive compounds. Addressing the valorization of citrus byproducts, this study investigates the extraction and antimicrobial properties of essential oils derived from sweet lime (*Citrus limetta*) peels. Essential oils were extracted via hydrodistillation, isolated, and initially characterized using thin-layer chromatography (TLC). Subsequent qualitative profiling was conducted using Gas Chromatography-Mass Spectrometry (GC-MS), which revealed a diverse array of bioactive compounds, with limonene identified as a predominant constituent. The antimicrobial efficacy of the extracted oil was evaluated against clinical isolates of *Salmonella typhi*, the causative agent of enteric fever, utilizing the agar well diffusion method to assess zones of inhibition. Given the significant antimicrobial properties of limonene, the extract demonstrated strong inhibitory effects. To our knowledge, this is the first study to establish the *in vitro* antibacterial activity of sweet lime essential oil against *Salmonella typhi*, highlighting its potential therapeutic application as a natural treatment alternative for typhoid fever.

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Graphical Abstract



1. Introduction

Global food systems currently face significant challenges regarding food loss and waste, with losses occurring at every stage of production, processing, distribution, and consumption. Approximately one-third of all food produced worldwide is ultimately discarded. The processing of fruits and vegetables alone generates substantial agro-industrial byproducts, comprising 25–30% of the total raw material. These byproducts—most commonly pomace, peels, rinds, and seeds—are frequently discarded as municipal solid waste. Currently, the world generates over 2 billion tonnes of municipal solid waste annually, a figure projected by the World Bank to surge to roughly 3.4 billion tonnes by 2050 [1]. Within this stream, food waste represents a massive fraction; the United Nations Environment Programme (UNEP) estimated that 1.05 billion tonnes of food waste were generated globally in 2022 alone [2]. However, rather than being mere waste, these fruit residues are highly rich reservoirs of valuable bioactive compounds, including polyphenols, carotenoids, vitamins, enzymes, and essential oils [3].

Citrus fruits represent one of the primary horticultural crops farmed globally and are the most traded horticultural product in the world. Driven heavily by markets such as North America, the per capita consumption of citrus has steadily increased over the past three decades. Consequently, the volume of citrus peel waste has surged. These peels are an exceptional source of essential oils (EOs). The extraction of EOs can be achieved through various advanced techniques, such as microwave-assisted extraction or liquid carbon dioxide, though traditional low- or high-pressure distillation using heated steam or boiling water remains the most common. The resulting essential oils comprise a complex mixture of diverse plant secondary metabolites, notably alkaloids, glycosides, terpenoids, flavonoids, and lignans [4].

Currently, there is a prominent consumer shift away from synthetic chemical agents and toward natural, plant-based products. While this transition offers potential health benefits, it simultaneously poses new environmental challenges. The large-scale industrial harvesting of medicinal plants required to meet this soaring

demand can inadvertently drive overexploitation and deforestation. To address this environmental paradox, utilizing agricultural waste for therapeutic formulations presents a highly sustainable alternative. Therefore, the present study aims to extract essential oil from organic municipal waste—specifically the peel of *Citrus limetta* (sweet lime)—and evaluate its antibacterial efficacy against *Salmonella enterica* serovar Typhi, the causative pathogen of enteric (typhoid) fever. By deriving this antibacterial formulation strictly from discarded citrus peels, this approach offers a dual benefit: mitigating agro-industrial waste and providing an eco-friendly, natural intervention against a severe foodborne and waterborne pathogen.

Despite advancements in sanitation and modern medicine, enteric fever caused by *Salmonella enterica* serovar Typhi remains a severe global public health burden, particularly in developing nations. The clinical management of typhoid is increasingly compromised by the rapid emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *S. Typhi* [5,6]. Conventional antibiotics, such as fluoroquinolones and cephalosporins, are frequently losing their efficacy, creating an urgent demand for novel, alternative antimicrobial agents. Plant-derived essential oils have garnered substantial attention in this context due to their broad-spectrum antimicrobial properties. The lipophilic nature of EO constituents, such as D-limonene and other monoterpenes typically found in *Citrus* species, allows them to easily penetrate the bacterial cell membrane, disrupting cellular integrity and neutralizing pathogens that resist standard antibiotics. While previous studies [7] have broadly evaluated the antimicrobial properties of citrus essential oils, the majority have focused on common Gram-positive bacteria or food spoilage organisms. To date, there is a paucity of targeted research addressing the efficacy of *Citrus limetta* essential oil specifically against the enteric fever pathogen, *Salmonella enterica* serovar Typhi. Unlike broader screenings, this study definitively establishes the *in vitro* inhibitory parameters of this specific horticultural waste derivative against this critical Gram-negative pathogen, highlighting its potential therapeutic application as a natural treatment alternative.

2. Materials And Method

2.1 Sample Collection and Preparation

Fresh peels of sweet lime (*Citrus limetta*) were utilized as the primary source material for essential oil extraction. The peel waste was procured from a local commercial juice vendor situated in Thiruvotriyur (Chennai, Tamil Nadu, India). To prevent external contamination, the discarded peels were collected in sterile polyethylene bags (Figure 1) and immediately transported to the laboratory.

Upon arrival, the peels were thoroughly washed with sterile distilled water to remove residual surface impurities. The white, inner spongy layer (albedo) was manually separated and discarded, as the outer colored layer (flavedo) harbors the primary essential oil glands. The isolated flavedo was sliced into uniform pieces to maximize surface area and naturally sun-dried for 48 to 72 hours. Once the moisture content was adequately reduced, the dried peel segments were pulverized into a fine powder using a standard laboratory blender and stored in airtight containers at room temperature until extraction.



Figure 1. Preparation of Citrus limetta samples. Fresh sweet lime peels were collected, manually separated to isolate the flavedo, and subsequently sun-dried to reduce moisture content prior to extraction.

2.2 Extraction and Recovery of Essential Oil

The extraction of essential oil from the prepared *C. limetta* peels was performed utilizing the hydrodistillation method. Briefly, 150 g of the powdered peel was weighed using a digital analytical balance and transferred into a round-bottom distillation flask, where an adequate volume of distilled water was added to completely submerge the plant material. The distillation unit was operated using a temperature-controlled heating mantle set to the boiling point of the solvent (Figure 2).



Figure 2. Essential oil extraction setup. The pulverized dried peel samples were subjected to hydrodistillation utilizing a temperature-controlled heating mantle and standard condensation apparatus



Figure 3. Isolation of essential oil. The resulting hydrodistillation distillate was filtered and transferred into a separating funnel to isolate the lighter upper lipidic layer from the denser lower aqueous phase.

Following distillation, the collected distillate was first passed through standard filter paper to remove any condensed particulate impurities. The filtrate was then transferred into a separating funnel to isolate the essential oil. Due to the difference in specific gravity, the mixture naturally resolved into two distinct phases: a denser lower aqueous layer and a lighter upper essential oil layer. The lower aqueous phase was carefully drained and discarded. The recovered pure essential oil was collected into sterile microcentrifuge tubes (Eppendorf) and stored in a refrigerator at 4 °C to prevent chemical degradation and potential fungal contamination prior to the antibacterial assays.

2.3 Phytochemical Profiling via Thin Layer Chromatography (TLC)

To verify the presence of active volatile constituents, the extracted essential oil was analyzed using Thin Layer Chromatography (TLC), following standard protocols for essential oil profiling [8,9]. Custom chromatographic plates were prepared by coating glass slides with a uniform slurry of fine silica gel, which were subsequently activated by drying in a hot air oven.

Aliquots of the extracted essential oil were carefully spotted near the baseline of the activated silica plates. The chromatograms were developed in a pre-saturated glass chamber containing a mobile phase of toluene and ethyl acetate in a 93:7 (v/v) ratio. The solvent was allowed to migrate until the solvent front reached approximately three-quarters of the plate height.

Following development, the plates were removed and air-dried at room temperature. Initial non-destructive visualization of the separated compounds was performed using a UV transilluminator. For further chemical characterization, the plates were derivatized using a vanillin-sulfuric acid spray reagent (prepared by dissolving 1 g of vanillin in 100 mL of ethanol, followed by the careful addition of 2 mL of concentrated sulfuric acid). Post-derivatization, the plates were gently heated to facilitate the colorimetric development of the spots, confirming the presence of active essential oil metabolites.

2.4 Qualitative Confirmatory Assays for Essential Oil

To qualitatively verify the lipidic composition and essential oil characteristics of the *C. limetta* extract, a series of standard biochemical assays were performed as detailed in (Table 1).

Table 1. Procedural methodology for the qualitative confirmation of essential oil.

Qualitative Test	Purpose	Procedure
Solubility Assessment	To evaluate hydrophobic behavior and dissolution properties.	Introduce a small aliquot of the extracted essential oil into separate glass test tubes containing equal volumes of distilled water, ethanol, and chloroform.
Translucent Spot Test	To visually verify the presence of persistent lipids.	Apply and gently rub a few drops of the extracted sample between the folds of standard cellulose filter paper. Examine against a light source to detect a transparent grease spot.
Baudouin Test	To detect the presence of specific unsaturated oils.	Add 5 mL of concentrated hydrochloric acid (HCl) and a 2% (v/v) furfural solution in alcohol to a test tube containing a small volume of oil. Agitate thoroughly and allow to stand at room temperature for 5 to 10 minutes.

2.5 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

To identify and quantify the volatile phytoconstituents present in the *C. limetta* essential oil, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted. The analysis was performed using a GC-2010 gas chromatograph coupled with a GCMS-QP2010 Plus mass spectrometer (Shimadzu). The carrier gas was maintained at a constant column flow rate of 1.00 mL/min, with a pressure of 53.5 kPa and a purge flow of 3.0 mL/min. The injection port temperature was set at 250 °C, operating in split mode with a split ratio of 1:50. The oven temperature program was initially set at 50 °C and held for 1.0 minute, followed by a linear temperature ramp of 10 °C/min up to a final temperature of 280 °C, where it was maintained for 2.0 minutes.

The mass spectrometer was operated with an ion source temperature of 200 °C and an interface temperature of 280 °C. Mass spectra were acquired in full scan mode with a mass-to-charge (m/z) range of 40 to 900, following a solvent cut time of 3.0 minutes. The identification of the volatile constituents was accomplished by comparing their mass spectra and retention times with those available in standard reference libraries [10,11].

2.6 In Vitro Antibacterial Assay and Determination of MIC

The antibacterial efficacy of the extracted *C. limetta* essential oil against a laboratory strain of *Salmonella enterica* serovar Typhi were evaluated utilizing the agar well diffusion method [12]. All microbiological procedures were strictly performed under aseptic conditions within a surface-sterilized laminar airflow cabinet.

The bacterial inoculum was prepared and standardized by adjusting the turbidity of the bacterial suspension to match a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Sterile Mueller-Hinton Agar (MHA) plates were prepared, and the standardized *S. Typhi* suspension was uniformly swabbed across the entire agar surface to ensure confluent bacterial growth.

Using a sterile cork borer, uniform wells of 6 mm were punched into the inoculated agar. Because essential oils are lipophilic and exhibit poor diffusion in aqueous agar, the extracted oil was serially diluted in Tween-80 to yield various working concentrations [12.5, 25, 50, and 100 µg/mL]. An exact volume of 50 µL of each concentration was meticulously introduced into the respective wells. The prepared Petri dishes were incubated at 37 °C for 24 hours. Following the incubation period, the antibacterial activity was quantified by measuring the diameter of the Zone of Inhibition (ZOI) in millimeters around each well. The MIC was defined and recorded as the lowest concentration of the essential oil that produced a visibly distinct zone of inhibition against the tested laboratory pathogen.

3. Results

3.1 Optimization of Hydrodistillation Temperature and Essential Oil Yield

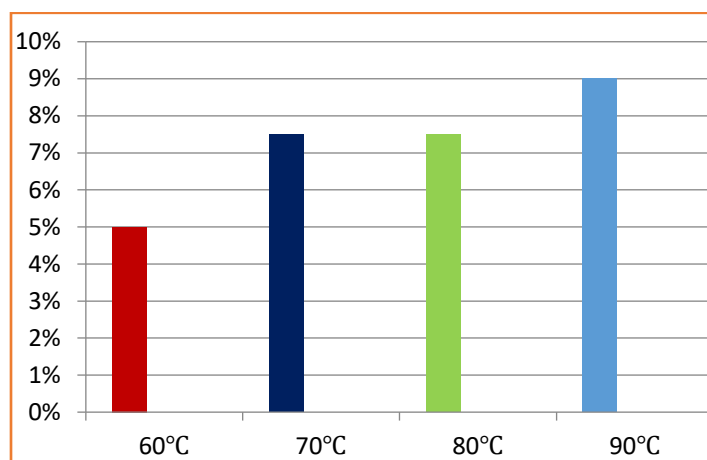


Figure 4. Optimization of hydrodistillation temperature. Extraction efficiency was evaluated across a temperature gradient, establishing 90 °C as the optimal parameter for maximum essential oil yield

The efficiency of essential oil extraction from *C. limetta* peels via hydrodistillation was systematically evaluated across a temperature gradient ranging from 60 °C to 90 °C to determine the optimal thermal parameters. The extraction yield demonstrated a clear temperature-dependent progression (Figure 4). Lower temperatures resulted in suboptimal recovery, with 60 °C yielding the lowest essential oil concentration. The yield increased progressively at 70 °C and 80 °C, before plateauing at its maximum.

The optimum temperature for the hydrodistillation process was definitively identified as 90 °C. Under these optimized conditions, a maximum yield of 12 mL of pure essential oil, corresponding to a 10% (v/w) extraction yield, was successfully extracted from 120 g of processed sweet lime peels over a continuous 160-minute distillation period using a standard aqueous solvent (Table 2). This represents a highly efficient recovery rate. Following phase separation, the isolated essential oil was refrigerated to maintain stability for subsequent phytochemical and antibacterial evaluations.

Table 2. Yield of Essential Oil

Sweet lime peels in grams	Time	Solvent	Oil Extracted (ml)
120g	160 minutes	Water	12ml

3.2 Phytochemical Screening via Thin Layer Chromatography (TLC)

The analytical assessment of the extracted *C. limetta* oil is a critical preliminary step prior to the advanced identification of individual volatile compounds. Initial phytochemical screening was performed using Thin Layer Chromatography (TLC) on silica gel plates to confirm the presence of active secondary metabolites.

Following solvent development, the chromatogram was derivatized using a vanillin-sulfuric acid spray reagent and gently heated. This procedure yielded distinct reddish-brown spots on the chromatogram (Figure 5). In accordance with established pharmacognostic protocols for essential oil analysis [8,9], the emergence of this specific reddish-brown coloration definitively confirms the presence of terpenoids and related volatile phytoconstituents. This qualitative result validated the successful extraction of complex bioactive metabolites, warranting further structural elucidation.

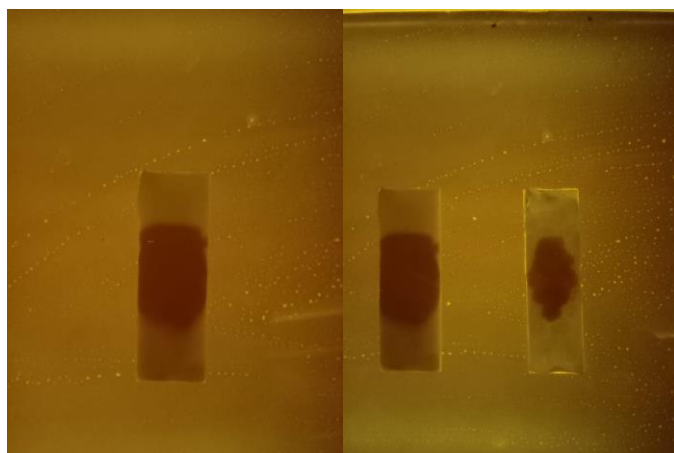


Figure 5. TLC chromatogram of *C. limetta* essential oil. The emergence of distinct reddish-brown spots following derivatization with vanillin-sulfuric acid reagent confirms the presence of terpenoids and volatile phytoconstituents.

3.3 Qualitative Confirmatory Tests for Essential Oil

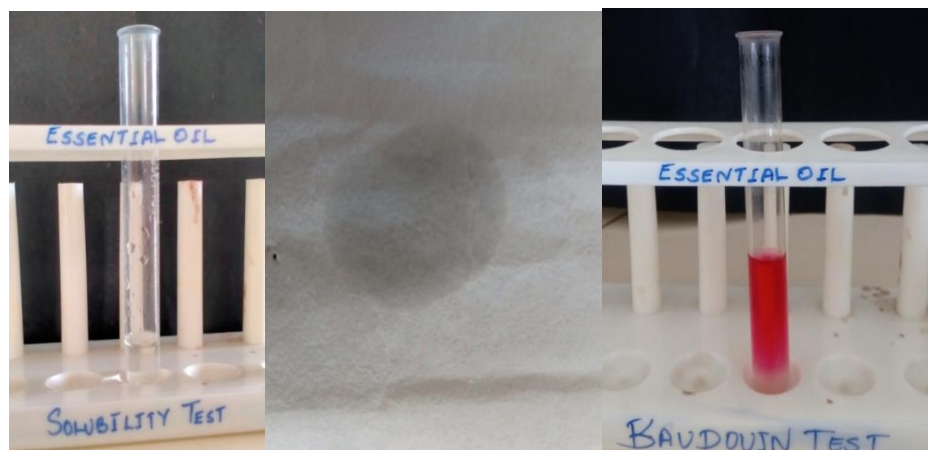


Figure 6. Qualitative confirmatory assays for the extracted essential oil, demonstrating characteristic lipidic behavior: (A) Solubility test highlighting hydrophobic phase separation, (B) Translucent spot test confirming persistent lipids, and (C) Baudouin test resulting in the characteristic coloration indicative of unsaturated oils.

The extracted *C. limetta* sample was evaluated using standard qualitative assays, which successfully confirmed its lipid and essential oil composition (Figure 6). In the solubility assessment, the extract exhibited characteristic hydrophobic behavior; it remained completely insoluble in the aqueous phase, forming a distinct superficial oily layer, yet demonstrated rapid and complete solubility in organic solvents (alcohol and chloroform). Furthermore, the translucent spot examination yielded a persistent transparent mark, corroborating the lipidic nature of the sample. Finally, the Baudouin assay resulted in the development of a characteristic rose-red coloration within 5 to 10 minutes, definitively confirming the presence of unsaturated oils within the extract.

3.4 Chemical Composition of *Citrus limetta* Essential Oil (GC-MS)

The chemical profile of the extracted *C. limetta* essential oil was extensively characterized utilizing Gas Chromatography-Mass Spectrometry. The resulting gas chromatogram revealed a highly complex mixture, displaying a total of 67 distinct peaks corresponding to various volatile phytoconstituents (Figure 7). The identified compounds, along with their respective retention times (RT) and relative peak area percentages, are detailed in (Table 3).

Quantitative analysis of the chromatogram demonstrated that the essential oil is predominantly composed of monoterpenes and sesquiterpenes. The most abundant constituent was identified as D-Limonene, representing a massive 71.27% of the total peak area, eluting at an RT of 5.42 minutes. Other major volatile compounds identified in significant concentrations included β -Pinene (3.84%, RT: 4.75 min), 1,6-Octadien-3-ol, 3,7-dimethyl (3.26%, RT: 6.06 min), and 1R- α -Pinene (3.17%, RT: 4.20 min).

Furthermore, trace amounts of significant bioactive sesquiterpenes and cyclic hydrocarbons were successfully detected and quantified, including Copaene (0.39%, RT: 8.67 min), Caryophyllene (0.20%, RT: 9.09 min), and α -Caryophyllene (0.11%, RT: 9.40 min). The comprehensive identification of these 67 peak fractions confirms the rich, multi-component nature of the hydrodistilled *C. limetta* peel extract.

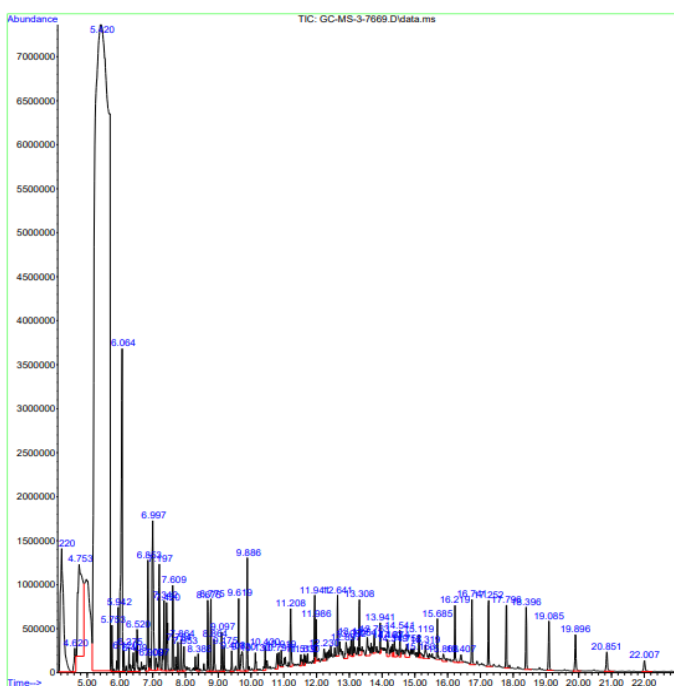


Table 3. Major phytochemicals identified in *Citrus limetta* essential oil via GC-MS analysis.

S.No	Peak No	RT	Area	Area%	Name Of The Compound	Quality
1.	1	4.20min	9216853	3.17	1R-.alpha.-Pinene	96
2.	2	4.62 min	843548	0.29	Bicyclo[3.1.0]hex-2-ene, 4-methy...	91
3.	3	4.75 min	1118545	3.84	beta.-Pinene	91
4.	5	5.42 min	2074950	71.27	D-Limonene	78
5.	5	5.75 min	540135	0.19	1,4-Cyclohexadiene, 1-methyl-4-	95
6.	5	5.94 min	1035844	0.36	(+)-4-Carene	97
7.	7	6.06 min	9502126	3.26	1,6-Octadien-3-ol, 3,7-dimethyl-	95
8.	8	6.12 min	231963	0.08	(E)-2-Butenoic acid, 2-(methylene	42
9.	9	6.27 min	384953	0.13	2-Cyclohexen-1-ol, 1-methyl-4-(1..	58
10.	10	6.40 min	439326	0.15	2-Cyclohexen-1-ol, 1-methyl-4-(1	95
11.	11	6.52 min	1143649	0.39	Cyclohexanol, 1-methyl-4-(1-meth.	96
12.	12	6.85 min	2293173	0.79	3-Cyclohexen-1-ol, 4-methyl-1-(1	95
13.	13	6.90 min	245158	0.08	1-(1-Butyny)cyclopentanol	46
14.	14	6.99 min	5218660	1.79	3-Cyclohexene-1-methanol, .alpha.	83
15.	18	7.42 min	1711336	0.59	2,6-Octadien-1-ol, 3,7-dimethyl-	95
16.	20	7.76 min	477914	0.16	1-Cyclohexene-1-carboxaldehyde, ..	95
17.	24	8.67 min	1140212	0.39	Copaene	98
18.	25	8.77 min	103900	0.36	1H-Cyclopropa[a]naphthalene, 1a,	94
19.	26	8.86 min	643341	0.22	Dodecanal	91
20.	27	9.09 min	591540	0.20	Caryophyllene	99
21.	29	9.40 min	312965	0.11	alpha.-Caryophyllene	99
22.	32	9.88 min	166801	0.57	Naphthalene, 1,2,3,5,6,8a-hexahy..	97
23.	37	11.20 min	1277118	0.44	Heptadecane	96
24.	47	13.30 min	1108685	0.38	Eicosane	98
25.	52	14.31 min	293832	0.10	Heneicosane	96

3.5 Morphological and Biochemical Characterization of the Bacterial Strain

To confirm the identity, viability, and purity of the laboratory strain of *Salmonella typhi* prior to the antibacterial assays, systematic macroscopic, microscopic, and biochemical evaluations were performed. Following 24 hours of aerobic incubation at 37 °C, cultivation in Nutrient Broth resulted in uniform turbidity. On Nutrient Agar plates, the bacteria developed into distinct grey-white, moist, circular colonies with smooth, convex surfaces measuring approximately 2 to 3 mm in diameter. Plating the strain on selective Salmonella Shigella (SS) Agar yielded characteristic colonies with black centers, confirming standard hydrogen sulfide production. Subsequent microscopic examination via Gram staining revealed the presence of Gram-negative bacilli (rods) (Table 4; Figure 8).



Figure 8. Morphological and biochemical identification of the *Salmonella enterica* serovar Typhi clinical isolate: A) Microscopic observation of Gram-negative bacilli following Gram staining; B) Uniform colonial growth on Nutrient Agar; C) Characteristic black-centered colonies on selective *Salmonella Shigella* (SS) Agar; and D) Positive Methyl Red and Citrate utilization on the IMViC biochemical panel.

Table 4. Cultural and morphological characteristics of the *S. Typhi* laboratory strain.

Growth Medium / Test	Observed Characteristics
Nutrient Broth	Uniform turbidity
Nutrient Agar	Grey-white, moist, and smooth convex surface
<i>Salmonella Shigella</i> (SS) Agar	Black colored colonies
Gram's Staining	Gram-negative rods

Further phenotypic validation was conducted utilizing the standard IMViC biochemical panel. The laboratory strain tested negative for Indole production and the Voges-Proskauer (VP) assay, while demonstrating positive reactions for the Methyl Red (MR) test and Citrate utilization (Table 5). This specific biochemical sequence (Indole -, MR +, VP -, Citrate +) serves as the definitive diagnostic pattern for *S. Typhi*, officially validating the strain for the subsequent well diffusion assays.

Table 5. IMViC biochemical profiling of the *S. Typhi* laboratory strain.

Biochemical Test	Result	Interpretation
Indole Test	Negative (-ve)	Inability to produce indole from tryptophan
Methyl Red (MR) Test	Positive (+ve)	Performs mixed-acid fermentation
Voges-Proskauer (VP) Test	Negative (-ve)	Absence of acetoin production
Citrate Utilization	Positive (+ve)	Utilizes citrate as a sole carbon source

3.6 In Vitro Antibacterial Activity

The antibacterial potency of the *C. limetta* essential oil was quantitatively evaluated against the verified laboratory strain of *Salmonella enterica* serovar Typhi. The pathogen exhibited significant, dose-dependent susceptibility to the extracted volatile oil (Figure 9).

To assess the efficacy gradient and determine the preliminary inhibitory threshold, varying volumes of the essential oil (25 μ L, 50 μ L, and 100 μ L) were introduced into the agar wells. The maximum antibacterial activity was recorded at the highest applied volume (100 μ L), which produced a prominent Zone of Inhibition (ZOI) measuring 1.9 cm (19 mm). A proportionate reduction in antibacterial activity was observed as the volume decreased, with the 50 μ L application yielding a ZOI of 1.2 cm (12 mm). The lowest tested volume of 25 μ L produced a clear ZOI of 0.9 cm (9 mm). Consequently, 25 μ L was recorded as the minimum effective volume under the tested agar diffusion parameters. Standard broth microdilution assays according to CLSI guidelines are required in future studies to ascertain the exact Minimum Inhibitory Concentration (MIC).

Table 6. Antibacterial activity (Zone of Inhibition) of Citrus limetta essential oil against Salmonella Typhi.

Essential oil (μ L)	Zone of Inhibition (cm)	Zone of Inhibition (mm) (mean $\pm$ SD)
25	0.9	9.0 $\pm$ 0.50
50	1.2	12.0 $\pm$ 1.00
100	1.9	19.0 $\pm$ 0.50

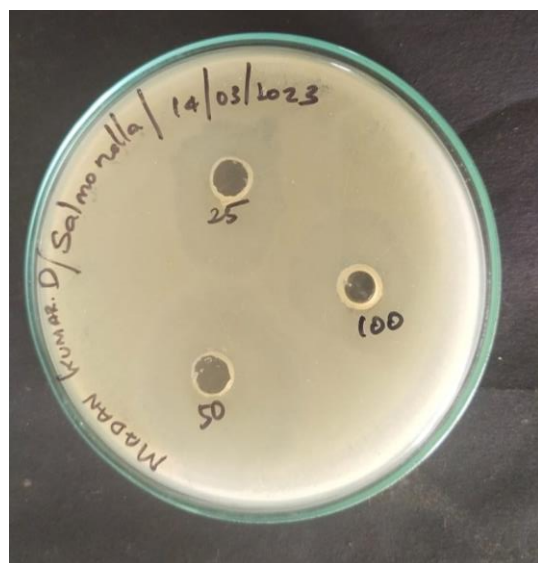


Figure 9. Antibacterial efficacy of *C. limetta* essential oil against *S. Typhi*. The agar well diffusion assay demonstrates dose-dependent zones of inhibition at applied volumes of 25 μ L, 50 μ L, and 100 μ L.

4. Discussion

The optimization of extraction parameters is a critical factor in maximizing the recovery of bioactive volatile compounds from agro-industrial waste. In the present study, the maximum yield of essential oil from *C. limetta* peels was determined to be 12 mL (from 120 g of raw material) at an optimized hydrodistillation temperature of 90 $^{\circ}$ C over 160 minutes. This yield is exceptionally efficient when compared to previous literature investigating citrus peel extractions. For instance, Tran et al. (2023) documented a maximal yield of 4% from citrus peels under high-temperature hydrodistillation (130 $^{\circ}$ C). The superior yield observed in the current study suggests that our specific,

moderate thermal parameters (90 °C) prevented the thermal degradation and evaporative loss of heat-sensitive volatile constituents, while the extended extraction time (160 min) maximized solvent penetration into the peel matrix.

Subsequent phytochemical screening via Thin Layer Chromatography successfully confirmed the presence of terpenoids, indicated by the characteristic reddish-brown spots following derivatization with vanillin-sulfuric acid reagent. This qualitative profile aligns closely with the essential oil compositions documented by [13]. To precisely elucidate this chemical composition, GC-MS analysis was conducted, revealing a highly complex profile of 67 distinct phytochemicals. The extract was overwhelmingly dominated by D-Limonene (71.27%), alongside significant concentrations of β -Pinene, 1R- α -Pinene, and the bicyclic sesquiterpene Caryophyllene. The identification of these high-quality volatile metabolites in significant quantities is consistent with previous chromatographic evaluations of citrus essential oils reported globally, including findings by [14].

Crucially, the present study establishes the robust *in vitro* antibacterial efficacy of this specific *C. limetta* extract against *Salmonella enterica* serovar Typhi, the primary causative agent of enteric fever. To the best of our knowledge, while citrus oils have been tested against various pathogens, this targeted efficacy specifically against laboratory strains of *S. Typhi* represents a highly significant addition to the existing literature. The antibacterial potency of essential oils is fundamentally linked to their lipophilic nature. The dominant monoterpene identified in our extract, D-Limonene, readily partitions into the lipid bilayer of the bacterial cell membrane. In Gram-negative bacteria such as *Salmonella*, this lipophilic interaction disrupts the structural integrity of the outer membrane and the cytoplasmic membrane, leading to an increase in permeability, the leakage of essential intracellular constituents, and ultimate cell death.

Our findings demonstrated a clear dose-dependent zone of inhibition, with a Minimum Inhibitory Concentration (MIC) established at 25 μ L. While previous studies by [15] and [16] have documented the inhibitory effects of essential oils primarily against Gram-positive and negative organisms (such as *Staphylococcus aureus* and *Escherichia coli*), our results conclusively prove that the concentrated phytochemicals in *C. limetta* oil are equally capable of breaching the robust outer membrane defenses characteristic of Gram-negative enteric pathogens. Ultimately, this study not only underscores the therapeutic potential of *C. limetta* essential oil as a natural, alternative antibacterial agent in the face of rising antimicrobial resistance, but it also demonstrates a highly sustainable methodology by utilizing discarded agro-industrial waste.

5. Limitations and Future Directions

While the findings of this study provide robust foundational evidence for the antibacterial potential of *C. limetta* essential oil, certain limitations must be acknowledged. First, the current investigation is strictly limited to *in vitro* assays. The complex physiological environment of a living host can significantly alter the bioavailability and stability of volatile phytochemicals. Therefore, subsequent studies must prioritize evaluating the cytotoxicity of this extract on standard mammalian cell lines, such as Vero cells, to determine the half-maximal cytotoxic concentration (CC50) and establish a safety profile. This critical safety validation, followed by *in vivo* efficacy trials using murine models, is necessary before proposing therapeutic applications. Secondly, the chemical composition of agro-industrial byproducts is inherently subject to geographical and seasonal variations; standardizing the extraction profile remains a challenge for future commercial scale-up. Finally, future research should incorporate advanced transcriptomic analyses to pinpoint the exact bacterial targets modulated by the essential oil constituents.

6. Conclusion

In conclusion, this study successfully demonstrates the dual potential of utilizing agro-industrial waste to develop novel, eco-friendly antimicrobial agents. The hydrodistillation of Citrus limetta peels, optimized at 90 °C, proved highly efficient, yielding a concentrated essential oil rich in bioactive volatile phytoconstituents. Advanced GC-MS analysis confirmed the presence of 67 distinct compounds, with the extract being overwhelmingly dominated by D-Limonene (71.27%), a monoterpene known for its lipophilic membrane-disrupting capabilities. Crucially, the extracted essential oil exhibited robust, dose-dependent *in vitro* antibacterial efficacy against *Salmonella enterica* serovar Typhi, demonstrating a notable Minimum Inhibitory Concentration (MIC) of 25 µL and a maximum zone of inhibition of 19 mm. These findings explicitly validate the therapeutic potential of C. limetta peel waste as a natural alternative to conventional antibiotics in combating enteric fever. By repurposing municipal organic waste into high-value antibacterial formulations, this approach offers a highly sustainable strategy to simultaneously mitigate environmental pollution and address the critical global health threat of antimicrobial resistance. While future investigations focusing on mammalian cytotoxicity and *in vivo* efficacy are required, this study establishes a strong foundational framework for the development of plant-derived, waste-sourced therapeutic interventions.

Competing interests

The author(s) declare no competing interests.

Authors' contributions

The research was conceived and planned by Madan Kumar D. & M. Nithiya Soundari. Madan Kumar D. performed experimental portion of the study and gathered information. Madan Kumar D. wrote the draft of the manuscript. M. Nithiya Soundari supervised the experimental work. The manuscript was read and modified by M. Nithiya Soundari.

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