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REVIEW ARTICLE

Effectiveness of *Citrus hystrix* Extracts in Antibacterial Therapy: A Systematic Review.

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Abstract

Citrus hystrix is a tropical medicinal plant known for its rich essential oils and its potential as a natural antibacterial agent. In this systematic review, the antibacterial efficacy of *Citrus hystrix* extracts is critically evaluated in relation to its phytochemical composition, providing an integrated analysis that links bioactive constituents with antimicrobial activity using 12 eligible studies published between 2005 and 2025 from Scopus, PubMed, Web of Science, ScienceDirect, and Google Scholar. Most studies investigated the essential oils of the peel or leaves and reported citronellal, limonene, and β-pinene as the main constituents. The extracts inhibited *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans*, and methicillin-resistant *S. aureus* (MRSA), with MIC values ranging from 3.12 to 12.5 mg/mL. Synergistic effects with antibiotics were also observed. However, the heterogeneity of extraction methods, the limited number of phytochemical profiles, and the reliance on in vitro assays limits the comparison between studies. Future work should focus on standardized extraction, comprehensive phytochemical profiling, detailed cytotoxicity assessment, and robust in vivo and clinical validation to enable therapeutic application of *Citrus hystrix* extracts.

Keywords: *Antibacterial activity, Citrus hystrix, essential oil.*

Introduction

The global rise in antimicrobial resistance (AMR) poses a critical threat to public health by undermining the effectiveness of standard antibiotics and complicating the treatment of infectious diseases. Common pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pneumoniae* have developed resistance to several classes of antibiotics, leading to longer periods of illness, higher hospitalization rates, and higher treatment costs. The overuse and misuse of antibiotics in healthcare, agriculture, and veterinary medicine have further accelerated the emergence of multidrug-resistant bacterial strains [1]. In response, natural products have gained attention as promising alternatives, with plant-derived essential oils (EOs) and extracts often exhibiting broad-spectrum antimicrobial activity and a relatively low tendency to develop resistance [2,3].

Citrus hystrix DC. (commonly known as kaffir lime) is a tropical plant from the Rutaceae family that is used for both culinary and medicinal purposes in Southeast Asia. In Malaysia, it is known as “limau purut” and in Thailand as “makrut”. Its aromatic leaves and peels are rich in EOs containing citronellal, limonene, and β-pinene [4,5], compounds that are well-documented for their antimicrobial properties. These bioactive molecules exert antibacterial effects through mechanisms such as disruption of bacterial cell membranes, inhibition of quorum sensing, and impairment of enzymatic function [2,5].

Pharmacological studies have also reported a wide range of biological activities of *C. hystrix* extracts, including antioxidant [4], anticancer [6], antifungal [7], and anticholinesterase effects [8], in addition to promising antibacterial activity [5,9,10]. However, previous studies and reviews have generally examined these aspects separately, either focusing on antibacterial efficacy or describing phytochemical composition without explicitly linking chemical constituents to biological activity. This divergence in research focus has limited the ability to establish clear relationships between phytochemical profiles and antibacterial effects across different studies. In addition, existing reviews are largely descriptive in nature and do not adopt a structured or comparative approach to evaluate how variations in extraction methods, chemical composition, and bacterial targets influence antibacterial outcomes. As a result, an integrated and systematic synthesis that critically examines the relationship between phytochemical composition and antibacterial activity remains insufficiently addressed in the current literature.

Therefore, this systematic review aims to critically evaluate and synthesize available evidence on the antibacterial activity of *C. hystrix* extracts in relation to their phytochemical composition. Specifically, this review qualitatively compares findings across studies by examining extraction methods, dominant bioactive compounds, and bacterial strains tested to identify patterns, consistencies, and key research gaps. To the best of our knowledge, this is among the first systematic reviews to explicitly integrate antibacterial activity with the phytochemical composition of *C. hystrix*. By linking chemical constituents with antimicrobial outcomes, this review provides a focused synthesis that supports future therapeutic and pharmaceutical development, while acknowledging the limitations of the currently available evidence.

Materials and Methods

Search strategy

A systematic literature search was conducted across five major electronic databases (Scopus, PubMed, Web of Science, ScienceDirect, and Google Scholar), selected to ensure comprehensive coverage of biomedical and multidisciplinary research relevant to *C. hystrix* and antibacterial activity. The search strategy combined controlled vocabulary and free-text terms, including (“*Citrus hystrix*” OR “kaffir lime”) AND (“essential oil” OR “plant extract”)

AND (“antibacterial” OR “antimicrobial”). The search string was adapted to the requirements of each database (e.g., title/abstract fields in PubMed and topic search in Web of Science). In addition, the reference lists of relevant review articles were screened to identify any additional eligible studies. To enhance reproducibility, the search process followed PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [11]. Only peer-reviewed, full-text articles published in English between 2005 and 2025 were included. The search was last updated in June 2025.

The number of records retrieved from each database is summarized in Table 1. Duplicate articles were removed prior to screening. In addition, the reference lists of the included articles were manually screened to identify further relevant studies.

Inclusion and exclusion criteria

Inclusion criteria:

- Original research articles that investigate the antibacterial activity of *C. hystrix* extracts (leaves, peels, fruits).
- Studies employing standardized microbiological assays (e.g., disc diffusion, minimum inhibitory concentration [MIC]).
- Studies reporting measurable antibacterial outcomes (e.g., zone of inhibition, MIC values).
- Peer-reviewed full-text articles published in English between January 2005 and June 2025

Exclusion criteria:

- Studies not investigating antibacterial activity (e.g., focused solely on antioxidant, anticancer, or antifungal properties).
- Review articles, conference abstracts, letters, patents, or other non-original research.
- Studies lacking standardized microbiological assays or measurable antibacterial outcomes (e.g., MIC or inhibition zones).
- Studies with insufficient experimental details or without an accessible full text.

Study selection and data extraction

All search results were exported to Microsoft Excel for duplicate removal and screening of titles and abstracts. Potentially eligible studies were retrieved in full text and independently assessed by two reviewers to ensure consistency and minimize selection bias. Any discrepancies were resolved through discussion and consensus. Data extraction was performed using a standardized approach, and a predefined data extraction form was used to ensure consistency across reviewers. The following information was collected from each study: author(s), year of publication, type of extract, extraction method, bacterial strains tested, antibacterial assay method (e.g., minimum inhibitory concentration (MIC) or disc diffusion), and key findings. The entire selection process was documented in accordance with PRISMA 2020 guidelines [11] and illustrated in Figure 1.

Records excluded (n = 396): The majority of excluded records were removed due to irrelevance to antibacterial activity, absence of primary experimental data, or failure to meet predefined inclusion criteria.

Full-text articles excluded (n = 4): These studies were excluded due to insufficient antibacterial outcome data or lack of methodological clarity.

Differences between the present review and previously published reviews may arise from the application of stricter inclusion criteria focusing on studies reporting measurable antibacterial outcomes.

Risk of bias assessment

A simplified quality assessment was conducted using criteria adapted from established tools, including the Joanna Briggs Institute (JBI) and Cochrane risk-of-bias frameworks. The assessment focused on key methodological domains, including clarity of methodology, specification of extract type, validation of bacterial strains, reporting of experimental replicates, and availability of phytochemical profiling (Table 2).

Based on these criteria, each study was qualitatively categorized as high, moderate–high, or moderate quality. This approach was considered appropriate given the heterogeneity of study designs and reporting formats across the included studies.

Results

Study selection overview

A total of 456 records were identified in five databases. After removing 44 duplicates, 412 records were screened, 16 were thoroughly assessed, and 12 studies were included according to PRISMA 2020 guidelines (Figure 1).

Characteristics of included studies

The included studies (n = 12), published between 2005 and 2025, demonstrate variability in extraction methods, bacterial targets, and reported outcomes. Most studies were conducted in Southeast Asia, with additional contributions from Bangladesh, Mauritius, and New Caledonia. Essential oils derived from peels and leaves were the most frequently investigated extracts, with steam distillation being the predominant extraction method (41.7%), followed by hydrodistillation, ethanol extraction, and green synthesis (Figure 2, Table 3). Across studies, antibacterial activity was most commonly evaluated against *Staphylococcus aureus* (8/12 studies, 66.7%) and *Escherichia coli* (7/12 studies, 58.3%), indicating a research focus on clinically relevant pathogens. Other bacterial species, including *Streptococcus mutans*, methicillin-resistant *S. aureus* (MRSA), *Shigella dysenteriae*, *Enterococcus faecalis*, and *Salmonella typhimurium*, were investigated less frequently (Figure 3).

Overall, a consistent trend across studies indicates that essential oils derived from *C. hystrix*, particularly from peels and leaves, exhibit stronger antibacterial activity compared to crude extracts, suggesting that the extraction method and concentration of bioactive phytochemicals play a key role in determining antibacterial efficacy.

Key findings

Essential oils, particularly those derived from peels, generally demonstrated antibacterial activity across multiple studies, especially against Gram-positive bacteria; however, the reported efficacy varied depending on extraction methods, plant parts, bacterial strains, and experimental conditions. Notably, activity against MRSA was reported in 3 studies (25.0%), suggesting potential relevance in antimicrobial resistance contexts.

Across the included studies, minimum inhibitory concentration (MIC) values varied depending on extract type and bacterial strain, generally indicating moderate to strong antibacterial activity. Lower MIC values (indicating higher potency) were more frequently associated with essential oils rich in monoterpenes such as citronellal, limonene, and β-pinene, supporting their role as key bioactive constituents.

These findings collectively demonstrate a relationship between phytochemical composition and antibacterial efficacy, although variability in experimental conditions limits direct quantitative comparison across studies.

Risk of bias

The quality of the studies ranged from moderate to high (Table 2). High-quality studies provided replicates, validated strains, and phytochemical profiles, which support reproducibility. In contrast, moderate quality studies often lacked replicates or phytochemical data, limiting comparability and highlighting the need for standardized reporting.

Phytochemical composition

Less than half of the studies reported complete phytochemical profiles (Table 4). Where reported, citronellal, limonene, and β-pinene, which are known to exert antibacterial effects via membrane disruption and inhibition of quorum sensing, consistently dominated. Their repeated demonstration supports their role as important bioactives, but inconsistent reporting limits comparison between studies and highlights the need for standardized phytochemical analysis.

Discussion

This systematic review emphasizes the broad antibacterial potential of *C. hystrix* extracts, especially the essential oils from the peel and leaves. Moderate to strong antibacterial activity was generally observed across studies; however, the reported efficacy varied depending on extraction methods, plant parts, bacterial strains, and experimental conditions. *S. aureus* and *E. coli* were the most frequently tested organisms [4,10,20]. Notably, the extracts were also effective against resistant strains such as MRSA [5,10], highlighting their potential importance in combating antimicrobial resistance (AMR). EOs, particularly from peels and leaves, were identified as the most active extracts across studies. *S. aureus* and *E. coli* were the most commonly susceptible bacterial strains, while reported MIC values generally ranged from approximately 3.12 to 12.5 mg/mL, depending on extract type and study conditions.

The antibacterial activity of *C. hystrix* is largely attributed to the dominant phytochemicals - citronellal, limonene, and β-pinene - which have been repeatedly identified in phytochemical analyses [4,5]. This integrated evaluation across studies highlights a consistent pattern linking these dominant monoterpenes with antibacterial efficacy, suggesting a structure-activity relationship that has been underexplored in previous reviews. By synthesizing both phytochemical and antibacterial data, this review provides a more comprehensive understanding of how chemical composition influences biological activity. These monoterpenes are known to disrupt bacterial membranes, impair enzymatic function, and inhibit quorum sensing [2,5,12], mechanisms that together explain the broad spectrum of inhibition. The convergence of phytochemical and antibacterial evidence strengthens confidence in these compounds as important bioactives, although the lack of a standardized phytochemical profile in many studies remains a major limitation.

Several studies have reported synergistic interactions between *C. hystrix* extracts and conventional antibiotics [15,16,20]. These findings are consistent with broader EO research in which natural compounds can restore or enhance the efficacy of antibiotics [2]. These results suggest a potential role for *C. hystrix* as an antibiotic adjuvant, providing a complementary strategy to curb resistance and reduce reliance on high-dose antibiotics.

Compared to other citrus species, *C. hystrix* demonstrates promising antibacterial activity; however, these comparisons are not based on direct, head-to-head studies and should therefore be interpreted with caution, as differences in extraction methods, bacterial strains, and assay conditions may influence the observed outcomes. For example, *Citrus limon* and *Citrus sinensis* show antibacterial effects, but often at higher MIC values and with a narrower spectrum of activity [23,24]. In addition to citrus fruits, plants such as *Azadirachta indica* (neem) and *Curcuma longa* (turmeric) have also been extensively studied for their antimicrobial properties. However, differences in reported applications, including synergistic formulations and novel delivery systems, may reflect variations in research focus and study design rather than direct comparative development [19,21]. Therefore, direct comparisons between studies remain limited, and further standardized investigations are required.

Despite these promising results, the current evidence base is dominated by in vitro studies, with only one in vivo model identified [17]. The lack of animal studies and clinical validation limits the translation of *C. hystrix* into therapeutic applications. None of the included studies systematically evaluated toxicity, which is a key obstacle to therapeutic translation.

Several methodological limitations were also identified. Comprehensive phytochemical profiling was not consistently reported, limiting the ability to correlate specific bioactive compounds with antibacterial efficacy. Furthermore, cytotoxicity assessments were incomplete or absent in many studies, restricting the evaluation of safety profiles. These limitations, together with the predominance of in vitro findings, highlight the need for standardized phytochemical characterization, rigorous toxicity evaluation, and well-designed in vivo and clinical studies to enhance the translational potential of *C. hystrix* extracts. In addition, the relatively small number of included studies (n = 12) may limit the generalizability of the findings and should be considered when interpreting the overall conclusions of this review.

It is also possible that positive results are overrepresented, as negative or invalid results are less frequently published. These gaps highlight the need for well-designed in vivo studies, comprehensive toxicity assessments, and clinical trials. Future work should also focus on standardized extraction methods and validated phytochemical analyses to ensure reproducibility and scalability in pharmaceutical or nutraceutical development.

Limitations of current evidence

This review is limited by the possible omission of regional, non-indexed studies and by the restriction to English-language publications. This restriction may have led to language bias, meaning that relevant regional studies may have been omitted. The considerable heterogeneity of extraction techniques, plant parts, and test methods prevented direct comparisons and precluded meta-analysis. In addition, inconsistent and incomplete phytochemical profiling across studies limited the ability to establish clear relationships between chemical composition and antibacterial activity, while the lack of comprehensive cytotoxicity evaluations further restricted safety assessment. The quality of the reports also varied. Several studies lacked replicates or phytochemical data, which limited reproducibility. The evidence base was predominantly derived from in vitro studies, with only one in vivo model and no clinical trials identified. Additionally, cytotoxicity assessments were limited or absent in most studies, further constraining conclusions regarding safety and therapeutic applicability. There may also be a publication bias, as studies with positive results are more likely to be published.

Conclusion

This review indicates that *Citrus hystrix* extracts, particularly essential oils from peels and leaves, exhibit promising antibacterial activity against both Gram-positive and Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). However, the available evidence is primarily derived from in vitro studies and is limited by methodological variability across studies. Citronellal, limonene, and β-pinene appear to be key bioactive compounds contributing to the observed antibacterial effects, with some evidence suggesting potential antibiotic synergy. To support clinical translation, future research should prioritize standardized extraction methods, comprehensive phytochemical profiling, toxicity assessment, and well-designed in vivo and clinical investigations.

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Table 1. Database search strategy and results.

Database	Search String (Keywords)	Date of Search	Number of Records Retrieved	Notes (Filters/Limitations)
Scopus	("*Citrus hystrix*" OR "kaffir lime") AND ("plant extract" OR "essential oil") AND ("antibacterial" OR "antimicrobial")	June 2025	268	English only; 2005–2025
PubMed	("*Citrus hystrix*"[Title/Abstract] OR "kaffir lime"[Title/Abstract]) AND ("essential oil" OR "plant extract") AND ("antibacterial" OR "antimicrobial")	June 2025	74	Humans/Animals; English; 2005–2025
Web of Science	("*Citrus hystrix*" OR "kaffir lime") AND ("essential oil" OR "plant extract") AND ("antibacterial" OR "antimicrobial")	June 2025	65	Articles only; English
ScienceDirect	("*Citrus hystrix*" OR "kaffir lime") AND ("essential oil" OR "plant extract") AND ("antibacterial" OR "antimicrobial")	June 2025	49	Journals only; 2005–2025
Google Scholar	"*Citrus hystrix*" OR "kaffir lime" AND "antibacterial" OR "antimicrobial"	June 2025	~300 (first 100 screened*)	Only peer-reviewed full-text is considered

Note:*Only the first 100 were screened due to relevance sorting and diminishing returns.

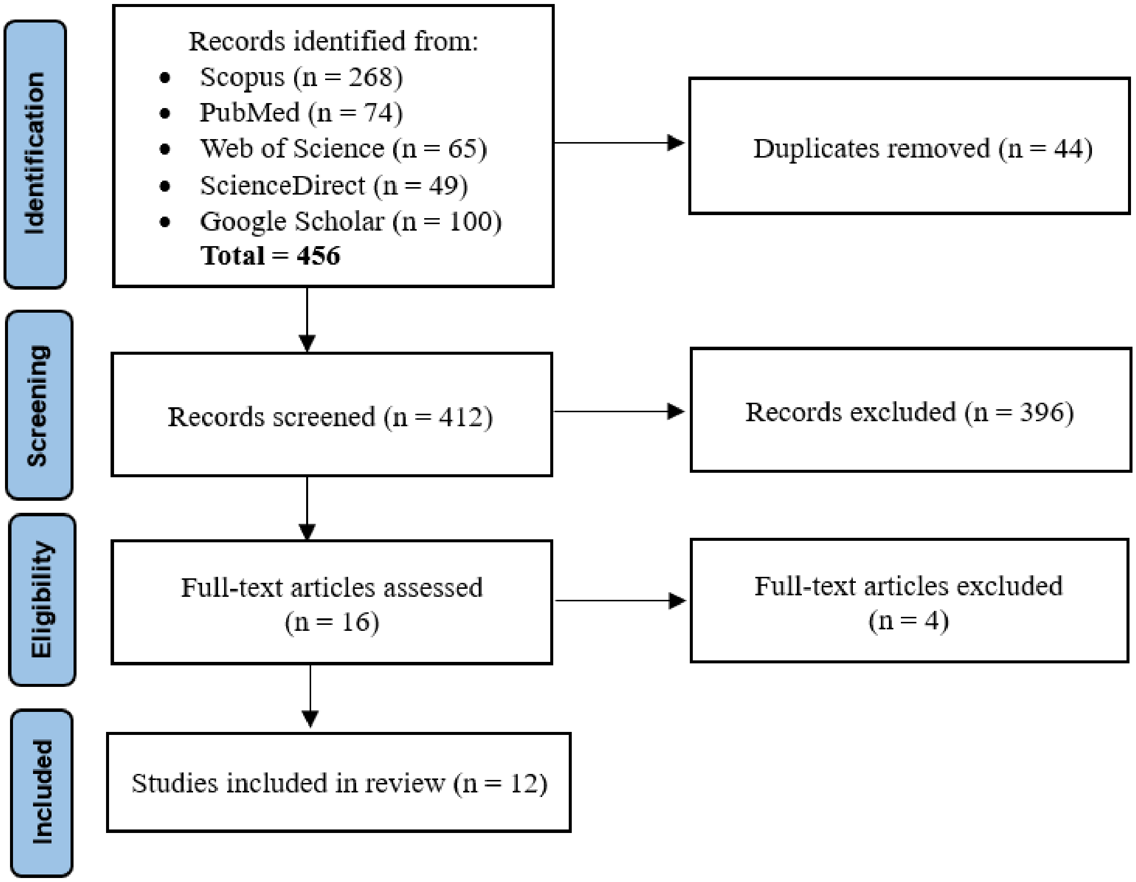

Figure 1. PRISMA 2020 flowchart of study selection for the systematic review. A total of 456 records were identified, 44 duplicates removed, 412 screened, 396 excluded, 16 assessed in full text, 4 excluded, and 12 included in the final review.

Table 2. Risk of bias and quality assessment of included studies.

Study (Author, Year)	Clear Methodology Reported	Extract Type Specified	Bacterial Strains Validated	Replicates Reported	Phytochemical Profile Provided	Overall Quality
Wannissorn et al., 2005 [12]	Yes	Yes	Yes	No	No	Moderate
Rahman et al., 2008 [13]	Yes	Partial (plant part unclear)	Yes	No	No	Low–Moderate
Waikedre et al., 2010 [5]	Yes	Yes	Yes	Yes	Yes	High
Kooltheat et al., 2016 [14]	Yes	Yes	Yes	Yes	Partial	Moderate–High
Aumeeruddy-Elalfi et al., 2016 [15]	Yes	Yes	Yes	Yes	Yes	High
Ulhaq et al., 2021 [17]	Yes	Yes	Yes	Yes	No	Moderate–High
Budiarto et al., 2021 [18]	Yes	Yes	Yes	Partial	Yes	Moderate–High
Mohideen et al., 2023 [4]	Yes	Yes	Yes	Yes	Yes	High
Le et al., 2023 [19]	Yes	Yes	Yes	Yes	Partial	High
Sreepian et al., 2023 [10]	Yes	Yes	Yes	Yes	Yes	High
Naibaho et al., 2024 [22]	Yes	Yes	Yes	Yes	Partial	High
Aini Khairunnisa et al., 2025 [20]	Yes	Yes	Yes	Yes	Yes	High

Quality assessment was conducted using criteria adapted from the Joanna Briggs Institute (JBI) and Cochrane risk-of-bias tools. Studies were evaluated based on methodological clarity, extract specification, bacterial strain validation, replicate reporting, and phytochemical profiling.

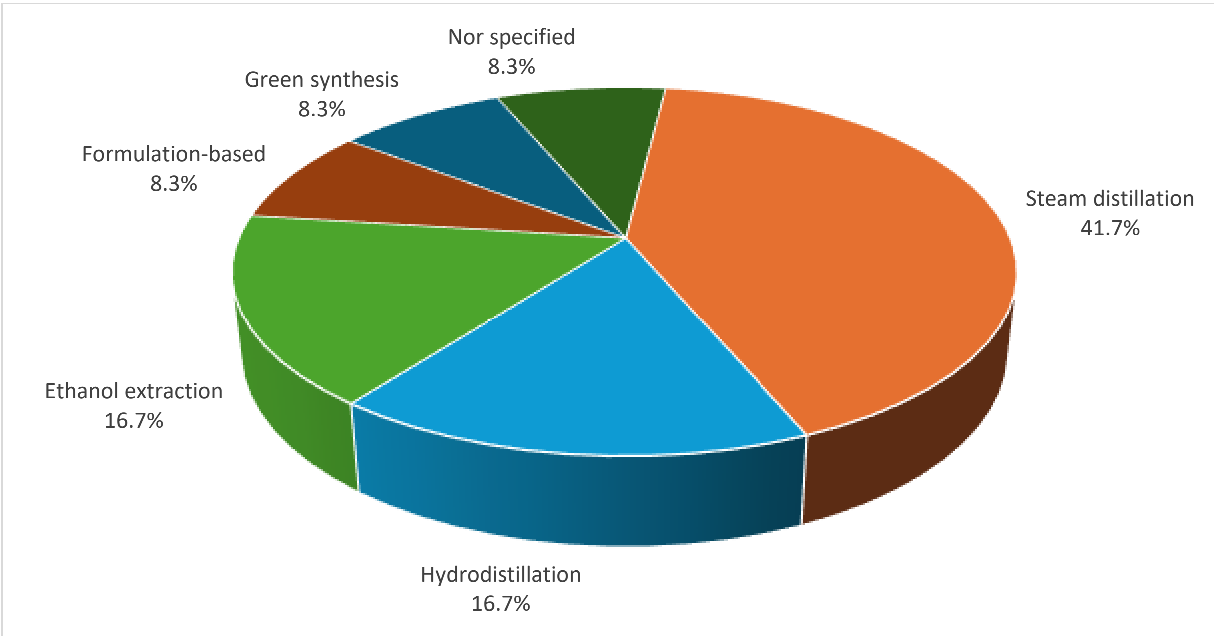

Figure 2. Distribution of extraction methods used in the included studies. Steam distillation was the most commonly used technique (41.7%), followed by hydrodistillation and solvent extraction. A minority of studies used formulation-based approaches, green synthesis, or did not specify the method.

Table 3. Summary of included studies on the antibacterial activity of *Citrus hystrix* extracts (2005–2025).

No.	Author (Year)	Country	Type of Extract	Extraction Method	Bacterial Strains Tested	Antibacterial Testing Method	Main Findings
1	Aini Khairunnisa et al. (2025) [20]	Indonesia	Peel essential oil	Not reported	*Escherichia coli*	In vitro and in silico assays	Synergistic with tetracycline; potent inhibition
2	Naibaho et al. (2024) [22]	Indonesia	Leaf essential oil	Steam distillation	*Staphylococcus aureus*, *Escherichia coli*	MIC, disc diffusion	High activity attributed to citronellal, limonene
3	Le et al. (2023) [19]	Vietnam	Peel essential oil in a lozenge	Formulation-based	*Multiple Gram-positive and Gram-negative strains*	Inhibition zone assay	Promising antibacterial activity in oral formulation
4	Sreepian et al. (2023) [10]	Thailand	Peel essential oil	Steam distillation	MRSA	Disc diffusion, MIC	Effective against multidrug-resistant and susceptible *S. aureus*.
5	Mohideen et al. (2023) [4]	Malaysia	Peel essential oil	Hydrodistillation	*Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Shigella dysenteriae*	Disc diffusion	Moderate-to-strong activity. (MIC: 3.12-12.5 mg/mL.
6	Ulhaq et al. (2021) [17]	Indonesia	Peel extract	Ethanol extraction	*Salmonella typhimurium*	MIC, in vivo (mouse model)	MIC ≈ 0.625% reduced load in vivo
7	Budiarto et al. (2021) [18]	Indonesia	Leaf essential oil	Steam distillation	*Staphylococcus aureus,*	MIC, disc diffusion	Moderate-to-strong

					Escherichia coli		antibacterial activity
8	Aumeeruddy-Elalfi et al. (2016) [15]	Mauritius	Essential oils blend	Steam distillation	*Escherichia coli*, *Staphylococcus epidermidis*	Disc diffusion, synergy test	Demonstrated antibiotic potentiation
9	Kooltheat et al. (2016) [14]	Thailand	Leaf extract	Not reported	*Streptococcus mutans*	Biofilm inhibition assay	Inhibited biofilm formation
10	Waikedre et al. (2010) [5]	New Caledonia	Essential oil	Distillation	Multiple Gram-positive and Gram-negative	MIC, MBC	Broad-spectrum antibacterial activity
11	Rahman et al. (2008)	Bangladesh	Plant extract	Not reported	Unspecified bacterial strains	Agar diffusion	Confirmed antimicrobial potential
12	Wannissorn et al. (2005) [12]	Thailand	Essential oil	Steam distillation	Unspecified bacterial strains	Disc diffusion, MIC	Among the most active medicinal plant extracts tested

MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration. ***Note***: Variability in extraction methods, assay conditions, and outcome reporting across studies limits direct comparison of antibacterial efficacy.

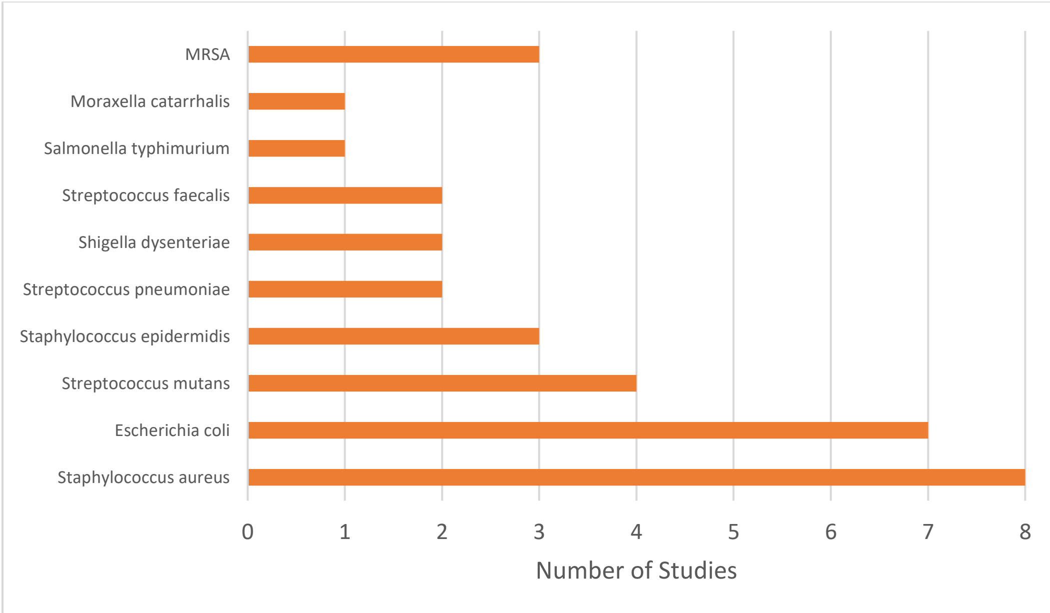

Figure 3. Frequency of bacterial strains tested in the included studies. *Staphylococcus aureus* and *Escherichia coli* were the most frequently tested species, followed by *Streptococcus mutans* and methicillin-resistant *S. aureus* (MRSA). The less frequently tested pathogens included *Shigella dysenteriae*, *Enterococcus faecalis*, and *Salmonella typhimurium*.

Table 4. Phytochemical reporting across included studies and antibacterial outcomes.

Study (Year)	Extract Type	Phytochemical Reporting	Major Phytochemicals (if reported)	Antibacterial Outcome (summary)
Wannissorn et al., 2005 [12]	Essential oil (Thai medicinal plants, including *C. hystrix*)	Partial	Not specified for *C. hystrix*)	Inhibition against common pathogens has been reported.
Rahman et al., 2008 [13]	Crude extracts (Bangladesh flora)	NR	Not reported	Antimicrobial activity screening; C. hystrix identified among active plants
Waikedre et al., 2010 [5]	Essential oil (peel/leaf)	Yes	Citronellal, limonene, β-pinene (representative)	Broad antibacterial activity, including resistant strains.
Kooltheat et al., 2016 [14]	Leaf extract	Partial	Not fully characterized (anti-biofilm focus)	Inhibits Streptococcus mutans biofilm formation
Aumeeruddy-Elalfi et al., 2016 [15]	Essential oil (multiple plants, including *C. hystrix*)	Yes	Representative terpene profiles reported	Antimicrobial activity with antibiotic potentiation.
Ulhaq et al., 2021 [17]	Ethanolic peel extract	NR	Not reported	Activity against Salmonella spp.; includes one in vivo model
Budiarto et al., 2021 [18]	Leaf essential oil	Partial	(Not fully detailed)	Antioxidant and antibacterial activity reported.
Le et al., 2023 [19]	Peel essential oil in lozenges	Partial	Menthol + *C. hystrix* EO (not fully specified)	Activity against pharyngitis-associated bacteria.
Sreepian et al., 2023 [10]	Essential oil	Partial	(Not fully detailed)	Potent activity against MRSA/MSSA clinical isolates.
Mohideen et al., 2023 [4]	Peel essential oil (hydro distillation)	Yes	Citronellal, limonene, β-pinene (representative)	MIC ~3.12–12.5 mg/mL against multiple pathogens.
Naibaho et al., 2024 [22]	Leaf essential oil	Partial	Citronellal, limonene (representative)	Strong antibacterial activity against S. aureus and E. coli
Aini Khairunnisa et al., 2025 [20]	Peel Essential oil (+ tetracycline synergy)	Partial	Limited mechanistic profile (in vitro/in silico)	Synergy against *E. coli*; antibiotic potentiation.

NR = Not reported; Partial = indicates that some phytochemical constituents were reported, but without comprehensive or quantitative profiling. Representative terpenes (e.g., citronellal, limonene, and β-pinene) are consistently identified as major bioactive compounds across the included studies.

References

[1] Siti, H. N., Mohamed, S., & Kamisah, Y. Potential therapeutic effects of *Citrus hystrix* DC and its bioactive compounds on metabolic disorders. *Pharmaceuticals,* 2022;15(3):320. doi.org/10.3390/ph15020167

[2] Saxena, G., & Kalra, S. S. Antimicrobial activity pattern of certain terpenoids. *International Journal of Pharma and Bio Sciences, 2011;2(1), 87–91*. Available from: https://ijpbs.net/abstract.php?article=NTQ= [cited 2025 December 6]

[3] Hien, T. T., Quyen, N. T. C., Truc, T. T., & Quan, P. M. Evaluate the chemical composition of kaffir lime (*Citrus hystrix*) essential oil using the classical method. *IOP Conference Series: Materials Science and Engineering, 2020 991*, 012014. doi:10.1088/1757-899X/991/1/012014

[4] Mohideen, M., Mahadi, N. N. S. J., Suhaimi, N. A. N., & Kamaruzaman, N. A. (2023). Antioxidant and antibacterial activities in kaffir lime (*Citrus hystrix*) essential oil extracted by the hydrodistillation method. In A. Ismail, F. N. Zulkipli, M. A. M. Daril, & A. Åchsner (Eds.), *Materials innovations and solutions in science and technology: With a focus on tropical plant biomaterials.* Springer. 2023;pp. 143–152.

[5] Waikedre, J., Dugay, A., Barrachina, I., Herrenknecht, C., Cabalion, P., & Fournet, A. Chemical composition and antimicrobial activity of the essential oils from New Caledonian *Citrus macroptera* and *Citrus hystrix*. *Chemistry & Biodiversity. 2010; 7*(4), 871–877. doi: 10.1002/cbdv.200900196.

[6] Tunjung, W. A. S., Cinatl, J., Michaelis, M., & Smales, C. M. Anticancer effect of kaffir lime (*Citrus hystrix* DC) leaf extract in cervical cancer and neuroblastoma cell lines. *Procedia Chemistry. 2015;14*, 465–468. doi.org/10.1016/j.proche.2015.03.062.

[7] Pumival, P., Tadtong, S., Athikomkulchai, S., & Chittasupho, C. Antifungal activity and the chemical and physical stability of microemulsions containing *Citrus hystrix* DC leaf oil. *Natural Product Communications.2020; 15*(12), 1–7. doi.org/10.1177_1934578X20957755

[8] Youkwan, J., Sutthivaiyakit, S., & Sutthivaiyakit, P. Citrusosides A–D and furanocoumarins with cholinesterase inhibitory activity from the fruit peels of *Citrus hystrix*. *Journal of Natural Products. 2010; 73*(11), 1879–1883. doi: 10.1021/np100531x

[9] Jiamboonsri, P., & Kanchanadumkerng, P. Influence of gallic acid and Thai culinary essential oils on antibacterial activity of nisin against *Streptococcus mutans*. *Advances in Pharmacological and Pharmaceutical Sciences, 2021*, 6628772. doi: 10.1155/2021/5539459

[10] Sreepian, P. M., Rattanasinganchan, P., & Sreepian, A. Antibacterial efficacy of *Citrus hystrix* (makrut lime) essential oil against clinical multidrug-resistant methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates. *Saudi Pharmaceutical Journal. 2023; 31*(6), 1094–1103. doi: 10.1016/j.jsps.2023.03.020.

[11] Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ.2021; 372*, n71. doi:10.1136/bmj.n71

[12] Wannissorn, B., Jarikasem, S., Siriwangchai, T., & Thubthimthed, S. Antibacterial properties of essential oils from Thai medicinal plants. *Fitoterapia. 2005 Mar;76(2):233-6. doi: 10.1016/j.fitote.2004.12.009.*

[13] Rahman, M. S., Rahman, M. Z., Wahab, M. A., Chowdhury, R., & Rashid, M. A. (2008). Antimicrobial activity of some indigenous plants of Bangladesh. *Dhaka University Journal of Pharmaceutical Sciences, 7*(1), 23–26. Available from: https://www.banglajol.info/index.php/JPharma/article/view/1469 [cited 2025 December 6]

[14] Kooltheat, N., Kamuthachad, L., Anthapanya, M., Samakchan, N., Sranujit, R. P., Potup, P., Ferrante, A., & Usuwanthim, K. Kaffir lime leaf extract inhibits biofilm formation by *Streptococcus mutans*. *Nutrition. 2016 Apr;32(4):486-90.* doi: 10.1016/j.nut.2015.10.010.

[15] Aumeeruddy-Elalfi, Z., Gurib-Fakim, A., & Mahomoodally, M. F. Chemical composition, antimicrobial, and antibiotic-potentiating activity of essential oils from 10 tropical medicinal plants from Mauritius. *Journal of Herbal Medicine. 2016; 6*(2), 88–95.doi:10.1016/J.HERMED.2016.02.002

[16] Agouillal, F., Taher, Z. M., Moghrani, H., Nasrallah, N., & El Enshasy, H. A. A review of genetic taxonomy, biomolecule chemistry, and bioactivities of *Citrus hystrix* DC. *Biosciences Biotechnology Research Asia.2017; 14*(1), 285–305. doi.10.13005/bbra/2446

[17] Ulhaq, Z. S., Hendyatama, T. H., Hameed, F., & Santosaningsih, D. Antibacterial activity of *Citrus hystrix* toward *Salmonella* spp. infection. *Enfermedades Infecciosas y Microbiología Clínica. 2021 Jun-Jul;39(6):283-286. doi: 10.1016/j.eimce.2020.05.016.*

[18] Budiarto, R., Poerwanto, R., Santosa, E., Efendi, D., & Agusta, A. Preliminary study on antioxidant and antibacterial activity of kaffir lime (*Citrus hystrix* DC) leaf essential oil. *Applied Research in Science and Technology.2021 1*(2), 58–65.doi.org/10.33292/areste.v1i2.8

[19] Le, N. T. M., Pham, S. T., Dang, K. D., Tran, Y. H., & Nguyen, T. N. N. Formulation of lozenges containing menthol and *Citrus hystrix* DC essential oil for pharyngitis support treatment. *Tropical Journal of Natural Product Research. 2023; 7*(10), 4233–4241.doi: 10.26538/tjnpr/v7i10.17

[20] Aini Khairunnisa, N., Yuandani, R., Raina Nasution, H., Nirmal, N., & Wira Septama, A. A synergistic interaction between *Citrus hystrix* peel essential oil and tetracycline and evaluation of their antibacterial mechanism of action *in vitro* and *in silico* against *Escherichia coli*. *Chemistry & Biodiversity. 2025 Jan;22(1):e202401291. doi: 10.1002/cbdv.202401291*.

[21] Hieu, N. H., Vinh An, T. T., Thu, N. M., Hanh, N. T., & Ngoc Hieu, N. T. Characterization and biological prospects of various calcined temperature-V_2O_5 nanoparticles synthesized by *Citrus hystrix* fruit extract. *Biochemical and Biophysical Research Communications.2024 Jul 30:719:150043. doi: 10.1016/j.bbrc.2024.150043. 719*, 150043.

[22] Naibaho, N. M., Safitri, A. S. M., Rudito, Popang, E. P., Syauqi, A., Saragih, B., Amirta, R., & Arung, E. T. (2024). Chemical composition and antibacterial activity of essential oil from kaffir lime (*Citrus hystrix* DC) leaves from East Borneo. *Food Research, 8*(1), 250–256. Available from: http://www.myfoodresearch.com/uploads/8/4/8/5/84855864/fr-2024-191_naibaho.pdf [cited 2025 December 6]

[23] Ramadan, M. F. Fruit oils: Chemistry and functionality. In A. Osman (Ed.), *Citrus oils* . 2029; pp. 521–540. Springer. doi.10.1007/978-3-030-12473-1_1

[24] da Silva, B. D., Bernardes, C. P., Pinheiro, F. P., Fantuzzi, E., & Roberto, C. D. Chemical composition, extraction sources, and action mechanisms of essential oils: Natural preservatives and limitations of use in meat products. *Meat Science2021 Jun:176:108463. doi: 10.1016/j.meatsci.2021.108463.*