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Investigating the Role of Kitchen Spices as Natural Antimicrobial Against *Clostridium Perfringens*

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Abstract

Background: This study aims to evaluate the antimicrobial potential of commonly used Indian spices against *Clostridium perfringens (C. perfringens)*, with particular focus on how solvent polarity influences extract potency. *C. perfringens* is an anaerobic, spore-forming bacterium responsible for severe gastrointestinal and soft-tissue infections, including gas gangrene. Due to increasing antibiotic resistance, there is a growing interest in plant-derived bioactive compounds as alternative antimicrobials.

Methods: This study investigated the anti-clostridial activity of Indian spice extracts Black pepper, Cinnamon, Clove, Coriander, Cumin, Star anise, and Turmeric which was prepared using Soxhlet extraction with solvents of varying polarity (methanol, ethanol, acetone, ethyl acetate, petroleum ether, and distilled water). Antibacterial activity was assessed using agar cup diffusion, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) assays following Clinical and Laboratory Standards Institute (CLSI) guidelines. Phytochemical profiling was performed through qualitative screening.

Results: Cinnamon, particularly its acetone extract, exhibited the strongest antibacterial activity (MIC=97.6 *µg/ml*; MBC=195.2 *µg/ml*). Clove and turmeric showed moderate inhibition, whereas petroleum-ether extracts of several spices demonstrated weak or bacteriostatic effects. Polar solvents extracted a broader diversity of phytochemicals, including terpenoids, flavonoids, and alkaloids. Statistical comparisons confirmed significant variation in antimicrobial efficacy depending on spice type and solvent.

Conclusion: These findings highlight cinnamon acetone extract as a promising natural antimicrobial candidate against *C. perfringens*, with potential applications in food safety and plant-based therapeutic development.

Keywords: Anaerobiosis, *Cinnamomum zeylanicum, Clostridium perfringens*, Coriandrum, Cuminum, Curcuma, Gas gangrene, Illicium, Methanol, Piper nigrum, Spices, Syzygium

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Introduction

Clostridial gas gangrene is a very serious life-threatening disease in which there is a rapid destruction of the tissue and production of gas accompanied by fever and systemic toxicity (1). The main causative agent of clostridial myonecrosis (gas gangrene) is *Clostridium perfringens (C. perfringens)*. This spore-forming, anaerobic pathogen is ubiquitous, found in soil where it can cause gangrene, and also as a common resident microflora in the intestines of humans and animals (2). *C. perfringens* can be divided into a few types based on the arsenal of toxins it has *i.e.,* Type A to type E. One of the major toxins is the Clostridial Alpha toxin also known as phospholipase C, this is the major virulent factor of the *Clostridium perfringens* and is encoded by the *plc* gene. The alpha toxin has the ability to form pores in the plasma membrane of the cells and also it can modulate the innate immune defense system (3).

Even though several antibiotics can inhibit the growth of *C. perfringens*, many of these drugs exhibit dose-dependent toxicity to human cells, and increasing reports of antibiotic resistance in *C. perfringens* further limit their long-term effectiveness (4), therefore it is of paramount importance for the development of anti-clostridial agents that are low in toxicity and high in efficacy against *C. perfringens*. Several traditional medicinal plants used across India possess antimicrobial and anti-inflammatory properties, and it is hypothesized that such phytochemicals may help inhibit the growth of *C. perfringens* and modulate the inflammation associated with clostridial myonecrosis. However, their therapeutic potential remains largely unexplored and requires systematic scientific validation. For Example, Garlic is used in Ayurveda for the treatment of gangrene and also garlic can inhibit NF-κB, thereby inhibiting the production of various cytokines involved in inflammatory responses (5).

Black pepper (*Piper nigrum L.),* has been utilized historically. In the past people have consumed black pepper as a traditional medication against several ailments such as digestion ailments, throat problems, inflammation, and also against bacterial infections (6). Black pepper can exhibit anti-*C. perfringens* activity, specifically inhibiting its growth in cooked chicken breast (7). With Generally Recognized as Safe (GRAS) status confirming its safety, cinnamon which is derived from the inner bark of Cinnamomum trees is widely used as a flavoring ingredient and is known for its anti-microbial, anti-inflammatory, and anti-cancer qualities (8,9). Cloves (*Eugenia caryophyllata*), which are frequently employed as flavoring agents, have strong anti-inflammatory, anti-microbial, and insecticidal qualities (10). Since ancient times, coriander, or *Coriandrum sativum*, has been valued for its culinary and therapeutic properties. It is effective against both gram-positive and gram-negative bacteria (11). *Cuminum cyminum*, often known as jeera, has long been used as a food preservative and digestive aid and it is also known for its potent antibacterial activity against both gram-positive and gram-negative bacteria (12,13). Studies have demonstrated the anti-microbial and anti-diabetic effects of star anise, which is known for its organoleptic properties. Its efficacy against MRSA USA 300 (14) and *C. perfringens* (15) in animal feed has been demonstrated. Indian culture has long embraced turmeric (*Curcuma longa*), a spice known for its antibacterial (16) a principal bioactive substance of turmeric (Curcuma longa L., anti-inflammatory, and anti-cancer properties. Curcumin, in particular, is well known for its ability to block bacterial biofilms and quorum sensing a principal bioactive substance of turmeric (Curcuma longa L. (17). Cloves (*Eugenia caryophyllata*), which are frequently employed as flavoring agents, have strong anti-inflammatory, anti-microbial, and insecticidal qualities that increase their usefulness outside of the kitchen (18).

The primary objective of this study is to systematically evaluate the antimicrobial efficacy of commonly used Indian spices against *C. perfringens,* and to determine how solvent polarity influences the extraction and activity of their phytochemical constituents. To achieve this, the authors employed a broad polarity range of solvents, beginning with non-polar petroleum ether, followed by mid-polar solvents such as ethyl acetate and acetone, and extending to highly polar solvents including methanol, ethanol and water. This gradient was intentionally selected because phytochemicals such as terpenoids, phenolics, flavonoids, alkaloids, and glycosides vary widely in their solubility profiles. Using solvents of differing polarity ensures more comprehensive extraction coverage and allows

the identification of specific solvent and spice combinations that yield the most potent anti-clostridial activity. Therefore, the authors hypothesized that the antibacterial efficacy of Indian spice extracts against *C. perfringens* varies according to solvent polarity, with polar and intermediates polarity solvents yielding more bioactive and potent constituents.

Materials and Methods

Bacterial strain and culture conditions

C. perfringens ATCC 13124 was procured from the American Type Culture Collection (ATCC) and cultured on Tryptic Soy Agar (Himedia) and Modified Reinforced Clostridial Medium (ATCC medium 2107, Himedia) under anaerobic conditions. Anaerobic incubation was performed using a 3.5-*liter* anaerobic jar and Gas Pack Kit (LE002a, Himedia) for 24-48 *hr*. For experimental assays, Brain Heart Infusion (BHI) broth and agar were used to maintain and propagate the organism. All microbial assays were carried out at the National Facility for Biopharmaceuticals, G.N. Khalsa College, Mumbai.

Plant material collection and processing

Commercially available spices including Cinnamon bark, Jeera (cumin), Black Pepper, Turmeric, Coriander seeds, Star Anise, and Clove were purchased from a local grocery store in Gandhi Market, Matunga, Mumbai. The spices were manually cleaned to remove any debris and then oven-dried at 40°*C*. The dried samples were ground to a coarse powder using a mechanical grinder and stored in airtight containers at 4°*C* until further use.

Preparation of plant extracts

Ten *g* of each powdered spice sample were packed into envelopes made from Whatman No.1 filter paper and subjected to exhaustive extraction using a Soxhlet apparatus with six different solvents: acetone, ethanol, methanol, ethyl acetate, petroleum ether, and distilled water. The extraction was continued until the siphoning solvent appeared colourless, indicating complete extraction. All extract concentrations were standardized based on the dry mass of the evaporated crude extract. After solvent removal, the dried residue was weighed, and working stock solutions were prepared by dissolving the crude extract in Dimethyl Sulfoxide (DMSO) or the respective solvent to achieve a final concentration of 10 *mg/ml*.

Anti-clostridial activity assays

Agar cup diffusion assay: Antibacterial activity was initially assessed using the agar cup diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2010. *C. perfringens* ATCC 13124 was cultured in BHI broth under anaerobic conditions at 37°*C* and standardized to a 0.5 McFarland standard (approximately 1.0×10^8 *CFU/ml*). The suspension was then diluted to 1.0×10^6 *CFU/ml* and mixed with molten BHI agar. Once solidified on Petri plates, wells were punched into the agar and filled with 100 μl of plant extract solutions. Each dried crude extract was reconstituted in DMSO to a final stock concentration of 10 *mg/ml*, and 100 μl of this solution was dispensed into each agar well for the agar cup diffusion assay. Chloramphenicol (30 μg) was used as the positive control antibiotic. The negative control consisted of DMSO without any plant extract. The solvent alone did not produce any inhibition zone, confirming that all observed antibacterial activity resulted from the extracts and not the solvent. Plates were incubated anaerobically at 37°*C* for 24 *hr*, and zones of inhibition were recorded.

Minimum inhibitory concentration (MIC): Minimum Inhibitory Concentration (MIC) was determined using a broth microdilution assay in 96-well microtiter plates. MIC testing was performed only for those extracts that exhibited measurable zones of inhibition in the agar cup diffusion assay. *C. perfringens* ATCC 13124 was adjusted to a final concentration of 1.0×10^6 *CFU/ml* in each well. Following anaerobic incubation, resazurin dye was added as a viability indicator; a change in color from blue to pink indicated bacterial growth. The MIC was recorded as the lowest concentration of the extract at which the blue color remained unchanged, indicating complete growth inhibition. Solvent-only wells containing DMSO behaved similarly to the growth control and showed no inhibitory effect, confirming that the solvent did not influence bacterial viability.

Minimum bactericidal concentration (MBC): To determine the Minimum Bactericidal Concentration (MBC), 10 μl from the MIC well and

the next two higher concentrations were streaked onto BHI agar plates supplemented with kanamycin (0.012 *mg/ml*) to ensure selectivity for ATCC 13124. Plates were incubated anaerobically for 24-48 *hr*. MBC was defined as the lowest concentration that produced no visible bacterial colonies.

Phytochemical screening

Qualitative phytochemical analysis of the extracts was conducted to detect the presence of terpenoids, flavonoids, glycosides, saponins, steroids, and alkaloids, following protocols described by (19) with minor modifications. Tests were based on colorimetric reactions and precipitation methods specific to each phytochemical group. Presence (+) or absence (–) was recorded for each extract-solvent combination.

Statistical analysis

All experimental measurements, including zone of inhibition, MIC, and MBC, were performed in triplicate. Data are presented as mean±standard deviation. A two-way Analysis of Variance (ANOVA) was conducted using GraphPad Prism to assess the effect of spice type and solvent polarity on antimicrobial activity. Significant differences among extract performances were further explored using Tukey's Honest Significant Difference (HSD) test ($p<0.05$). For the binary phytochemical screening data, Chi-square tests were also performed in GraphPad Prism to examine associations between phytochemical presence and extraction solvent.

Results

Antibacterial assay

The antibacterial activity of spice extracts—including black pepper, clove, cumin, coriander, turmeric, star anise, and cinnamon—was evaluated using six different solvents (methanol, ethanol, acetone, ethyl acetate, petroleum ether, and distilled water) against *C. perfringens* ATCC 13124 (Figures 1 and 2, Table 1). The two-way ANOVA showed highly significant main effects of spice type [$F(4,50)=938.77$, $p=2.37\times10^{-46}$] and solvent [$F(5,50)=21.72$, $p=1.73\times10^{-11}$], as well as a strong spice × solvent interaction [$F(20,50)=93.71$, $p=9.75\times10^{-33}$] on the zone of inhibition. These findings confirm that both the botanical origin of the extract and the solvent used play critical roles in determining antibacterial efficacy.

Subsequent Tukey's HSD post-hoc analysis identified several statistically significant pairwise differences,

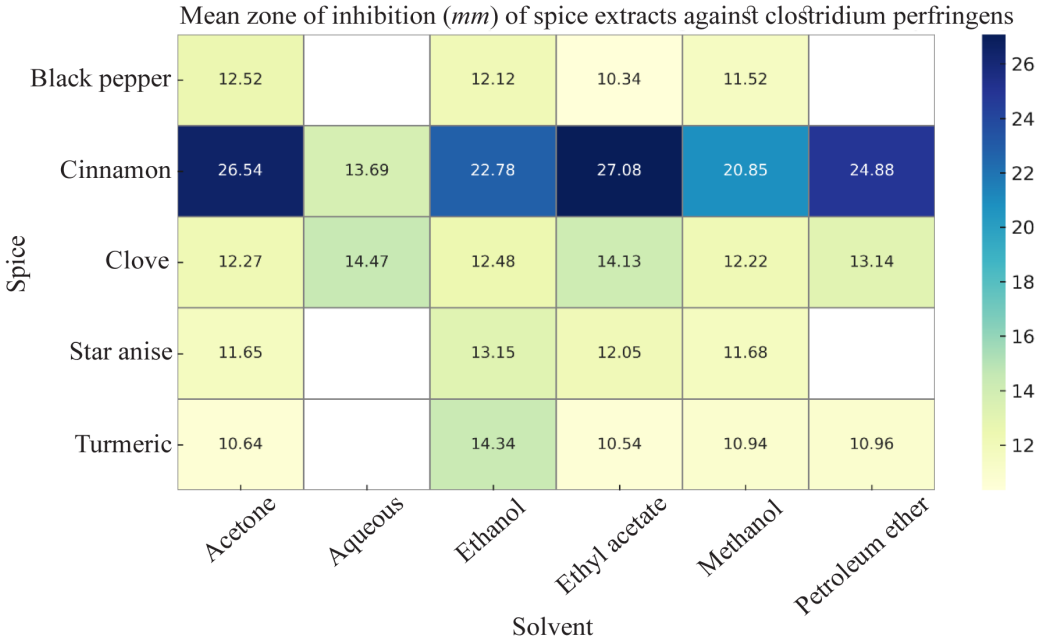


Figure 1. Heatmap representing the mean zone of inhibition (*mm*) of various spice extracts prepared using different solvents against Clostridium perfringens ATCC 13124. The data reflects the average of triplicate assays. Notably, clove and cinnamon extracts demonstrated the highest antibacterial activity across multiple solvents, while cumin and coriander showed no inhibitory effect. The differential response emphasizes the role of both spice type and extraction solvent in determining antimicrobial efficacy.

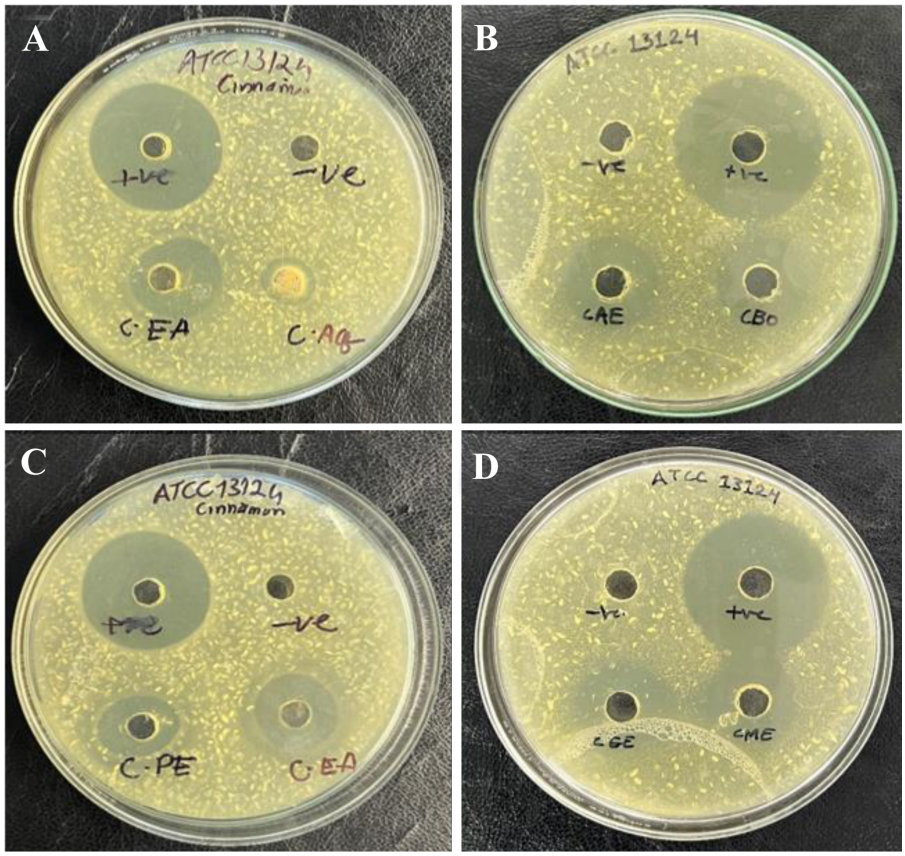


Figure 2. Representative images showing the antibacterial activity of various solvent extracts of cinnamon bark against Clostridium perfringens (ATCC 13124) using the agar well diffusion method. A) Aqueous (C-AQ) and ethyl acetate (C-EA) extracts; B) Acetone extract (C-AE) and commercial cinnamon bark oil (CBO); C) Petroleum ether (C-PE) and ethyl acetate (C-EA) extracts; D) Ethanol (C-EE) and methanol (C-ME) extracts. +VE: Positive control (antibiotic); –VE: Negative control (solvent). Clear zones around wells indicate inhibitory activity against *C. perfringens*.

Table 1. Mean zone of inhibition (*mm*) of spice extracts against Clostridium perfringens

Spice	Acetone	Aqueous	Ethanol	Ethyl acetate	Methanol	Petroleum ether
Black pepper	12.52	-	12.12	10.34	11.52	-
Cinnamon	26.54	13.69	22.78	27.08	20.85	24.88
Clove	12.27	14.47	12.48	14.13	12.22	13.14
Coriander	-	-	-	-	-	-
Cumin	-	-	-	-	-	-
Star anise	11.65	-	13.15	12.05	11.68	-
Turmeric	10.64	14.34	10.54	10.54	10.94	10.96

particularly between highly effective extracts (such as cinnamon-acetone and clove-ethyl acetate) and less effective ones (such as turmeric or black pepper in certain solvents). For example, cinnamon extracted with acetone exhibited a significantly higher zone of inhibition compared to black pepper extracted with ethyl acetate ($p<0.001$), further reinforcing the superior antimicrobial potential of certain spice-solvent combinations.

Consistent with the statistical results, clove and cinnamon extracts showed the highest antibacterial activity, with significant inhibition zones across all solvent systems—including aqueous extracts—indicating the robust and solvent-independent

nature of their bioactive constituents. Black pepper and star anise extracts exhibited moderate activity, significantly outperforming turmeric in multiple solvent comparisons. On the other hand, cumin and coriander extracts failed to demonstrate any antibacterial activity, showing no zone of inhibition across all solvent systems.

The MIC and MBC of selected Indian spice extracts were systematically evaluated against *C. perfringens* ATCC 13124 using various organic and aqueous solvents. The results, presented in figure 3 and table 2 (MIC) and figure 4 and table 3 (MBC), provide critical insight into the antibacterial potency and efficacy of each extract-solvent combination. For MIC values, two-way ANOVA indicated highly significant main effects of spice type [$F(4,52)=2670.13$, $p=1.77\times10^{-59}$] and solvent [$F(5,52)=227.63$, $p=4.49\times10^{-34}$], as well as a strong spice×solvent interaction [$F(20,52)=1032.12$, $p=1.75\times10^{-60}$]. For MBC values, spice type [$F(4,50)=106.43$, $p=9.58\times10^{-19}$], solvent [$F(5,50)=357.36$, $p=2.44\times10^{-30}$], and their interaction [$F(20,50)=448.73$, $p=1.45\times10^{-48}$] were likewise highly significant, highlighting the importance of solvent-spice synergy.

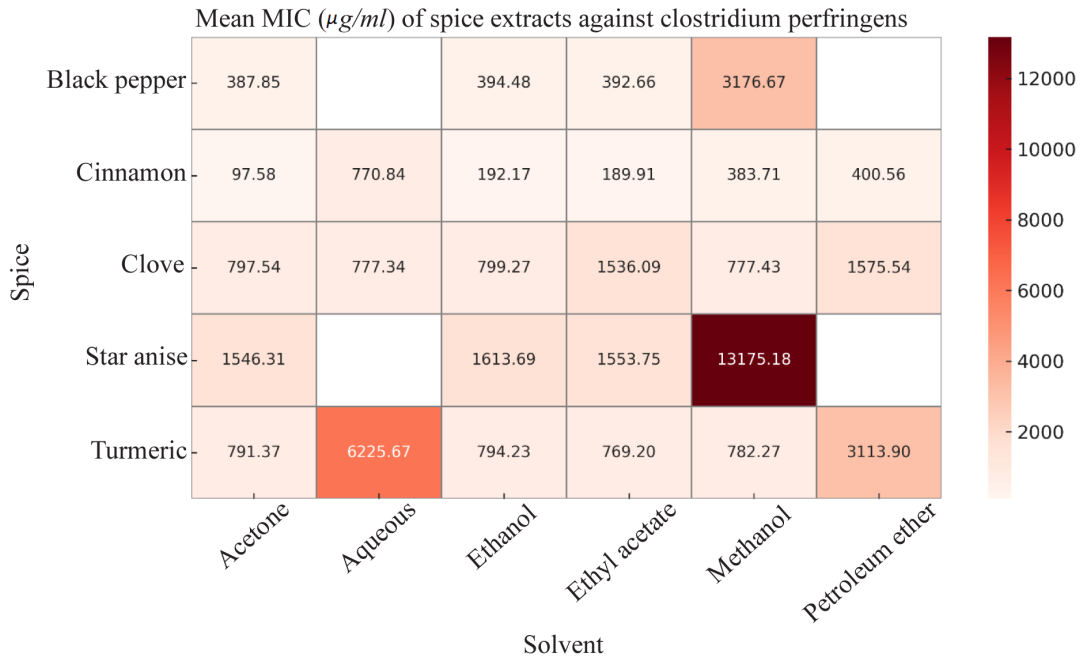


Figure 3. Heatmap depicting the mean Minimum Inhibitory Concentration (MIC, µg/mL) of various Indian spice extracts prepared using different solvents against C. perfringens ATCC 13124. Data represents the average of triplicate measurements. Lower MIC values indicate higher antimicrobial potency. Clove and cinnamon extracts showed superior inhibitory activity across multiple solvents, whereas cumin and coriander exhibited no activity.

Table 2. Mean minimum inhibitory concentration (MIC, µg/mL) of spice extracts against Clostridium perfringens

Spice	Acetone	Aqueous	Ethanol	Ethyl acetate	Methanol	Petroleum ether
Black pepper	387.85	-	394.48	392.66	3176.67	-
Cinnamon	97.58	770.84	192.17	189.91	383.71	400.56
Clove	797.54	777.34	799.27	1536.09	777.43	1575.54
Coriander	-	-	-	-	-	-
Cumin	-	-	-	-	-	-
Star Anise	1546.31	-	1613.69	1553.75	13175.18	-
Turmeric	791.37	6225.67	794.23	769.2	782.27	3113.9

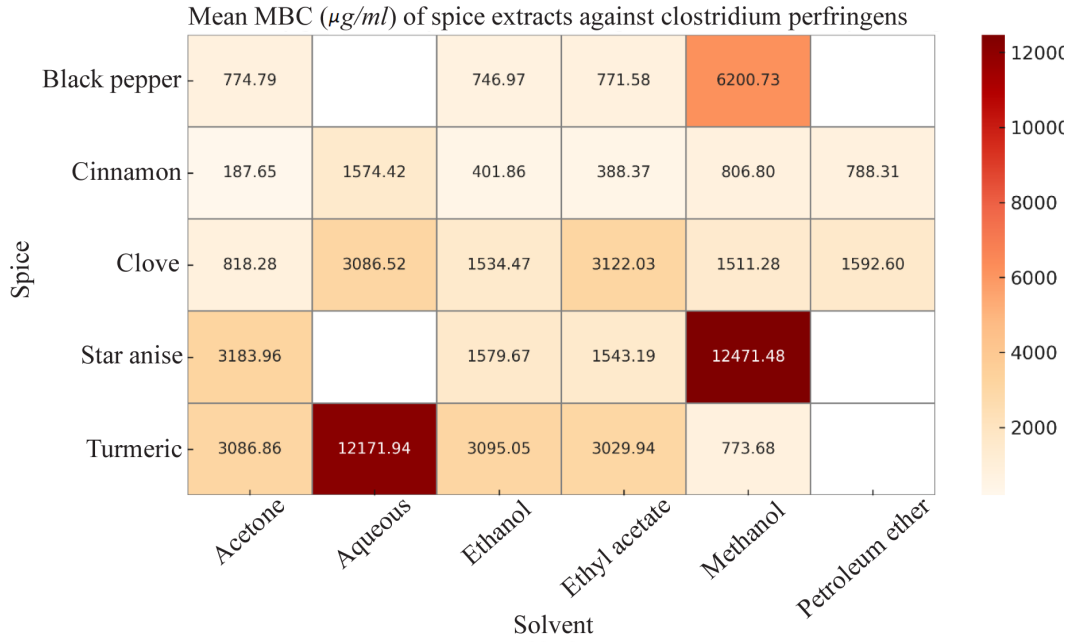


Figure 4. Heatmap showing the mean Minimum Bactericidal Concentration (MBC, *µg/ml*) of Indian spice extracts obtained using different solvents against C. perfringens ATCC 13124. Values are averaged from triplicate experiments, with at least one original replicate retained. Cinnamon and clove demonstrated strong bactericidal properties, while turmeric and black pepper required higher concentrations for bactericidal effects. Cumin and coriander showed no bactericidal activity.

Table 3. Mean minimum bactericidal concentration (MBC, µg/mL) of spice extracts against Clostridium perfringens

Spice	Acetone	Aqueous	Ethanol	Ethyl acetate	Methanol	Petroleum ether
Black Pepper	774.79	-	746.97	771.58	6200.73	-
Cinnamon	187.65	1574.42	401.86	388.37	806.8	788.31
Clove	818.28	3086.52	1534.47	3122.03	1511.28	1592.6
Coriander	-	-	-	-	-	-
Cumin	-	-	-	-	-	-
Star Anise	3183.96	-	1579.67	1543.19	12471.48	-
Turmeric	3086.86	12171.94	3095.05	3029.94	773.68	-

Preliminary screenings ruled out coriander, cumin, and the aqueous and petroleum ether extracts of black pepper and star anise due to their lack of observable antibacterial activity, and these were therefore not included in MIC/MBC testing. Among the active extracts, black pepper exhibited potent antimicrobial activity when extracted using ethanol, acetone, and ethyl acetate, each yielding a MIC of 390.6 *µg/ml* and an MBC of 781.25 *µg/ml*, suggesting effective extraction and bioactivity of compounds in these solvents.

Clove extracts showed broad-spectrum antibacterial potential. Particularly, the acetone extract demonstrated strong bactericidal action (MIC and MBC both at 781.25 *µg/ml*), while the aqueous extract yielded a significantly higher MBC (3125 *µg/ml*), likely due to reduced solubility of key bioactive compounds in water. These findings were further substantiated by Tukey's HSD, which revealed significant differences between aqueous and organic extracts ($p<0.05$).

Turmeric's methanolic extract emerged as the most

effective, with an MBC of 781.25 *µg/ml*. In contrast, extracts using acetone, ethanol, and ethyl acetate showed moderate bactericidal activity (MBC=3125 *µg/ml*), and the petroleum ether extract exhibited a bacteriostatic effect, inhibiting but not killing the pathogen. The aqueous extract displayed a notably high MBC (12,500 *µg/ml*), reinforcing the pattern of poor aqueous solubility observed across other spices as well.

Cinnamon extracts demonstrated the highest antimicrobial potency overall, especially when extracted in acetone. The MIC of cinnamon-acetone was 97.6 *µg/ml*, and the MBC was 195.2 *µg/ml*, the lowest among all tested samples, indicating strong inhibitory and bactericidal activity. Organic solvent extracts of cinnamon (methanol, acetone, and ethyl acetate) consistently showed low MICs (195.3-390.6 *µg/ml*) and MBCs (390.6-781.25 *µg/ml*), positioning cinnamon as a standout candidate for antimicrobial development.

Conversely, star anise methanolic extract displayed the highest MIC and MBC values (12,500 *µg/ml*), signifying poor efficacy and the need for high concentrations to achieve microbial inhibition and killing. This is in contrast to its performance in other solvents like ethanol and ethyl acetate, where lower MBCs were observed.

The heatmaps visually reinforced these trends, clearly distinguishing low MIC and MBC zones (indicative of strong activity) in cinnamon and clove extracts across solvents, and highlighting weak or ineffective regions for aqueous and non-polar extracts of turmeric and star anise.

Phytochemical screening

The phytochemical analysis summarized in figure 5 revealed the presence of various bioactive compounds across different Indian spice extracts, depending

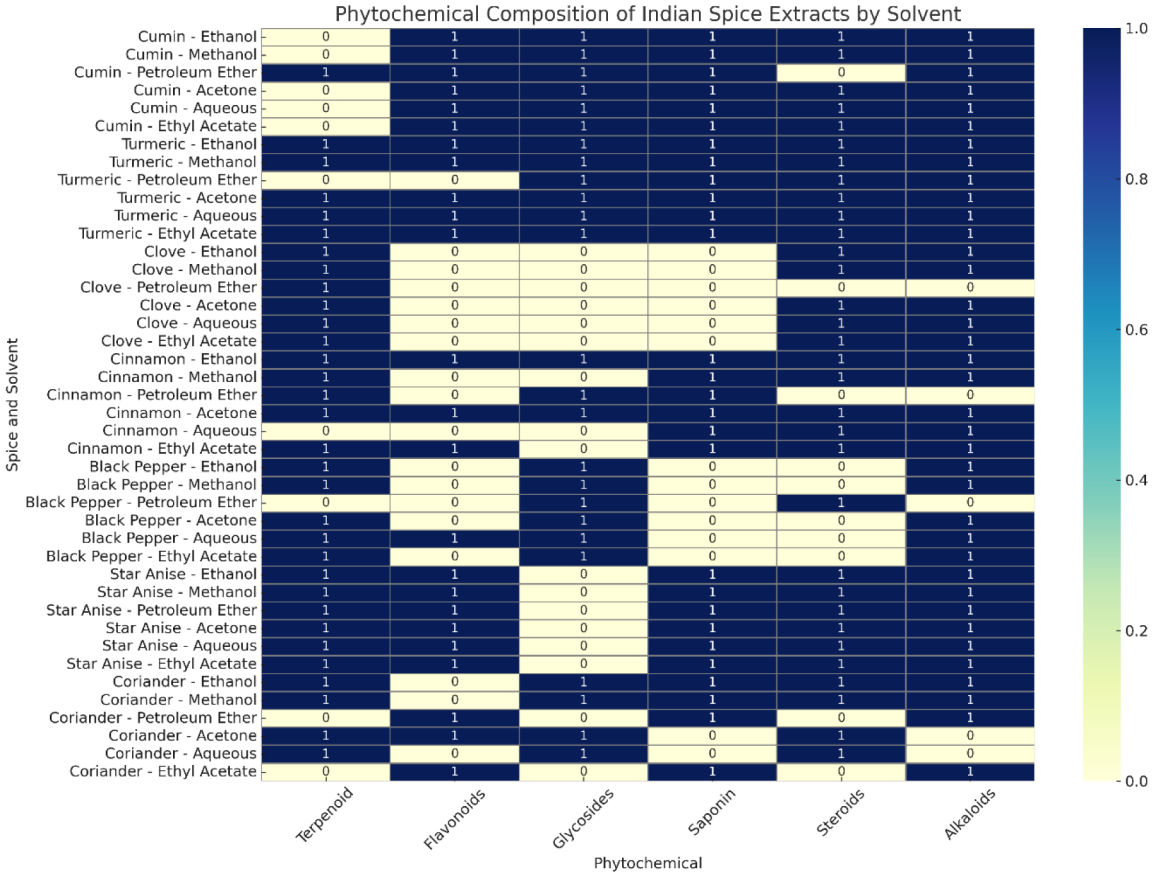


Figure 5. Heatmap depicting the presence (+) and absence (–) of major phytochemical groups—including terpenoids, flavonoids, glycosides, saponins, steroids, and alkaloids—in Indian spice extracts prepared using different solvents. The presence of each compound class is coded as 1 (blue) and absence as 0 (white). Notable variations were observed based on both the spice and solvent, highlighting the selective solubility of phytoconstituents.

significantly on the type of solvent used for extraction. Each spice demonstrated a unique phytochemical profile, with notable variation influenced by solvent polarity. The compounds screened included terpenoids, flavonoids, glycosides, saponins, steroids, and alkaloids. Polar solvents such as ethanol and methanol consistently exhibited broad extraction capabilities, efficiently isolating a wide range of phytochemicals across most spices. In contrast, the non-polar solvent petroleum ether was more selective, effectively extracting terpenoids in spices like cumin, cinnamon, and star anise, and occasionally steroids and alkaloids, though overall extraction was limited. Mid-polar solvents such as acetone and ethyl acetate provided intermediate extraction efficiency and were particularly effective in extracting a broad range of compounds in cinnamon, coriander, and clove. Specifically, cumin extracts contained all tested phytochemicals except terpenoids, which were found only in the petroleum ether extract. Turmeric showed a wide range of phytochemicals in all solvents except petroleum ether, where terpenoids and flavonoids were absent. Clove extracts lacked flavonoids and glycosides but consistently showed terpenoids, steroids, and alkaloids. Cinnamon was among the most phytochemically rich spices, with saponins present in all extracts, and ethanol and acetone extracting nearly the complete spectrum. Black pepper extracts were rich in glycosides, terpenoids, and alkaloids, with petroleum ether showing poor extraction performance. Star anise extracts demonstrated the presence of all phytochemicals except glycosides across all solvents, while coriander showed better phytochemical presence in ethanol and methanol extracts. These results collectively confirm the critical role of solvent polarity in extracting specific classes of bioactives and highlight the chemical diversity of Indian spices, offering valuable insights for their potential pharmacological applications.

Discussion

The current study provides a comprehensive evaluation of the antibacterial efficacy of various Indian spice extracts against *C. perfringens* ATCC 13124, with a particular focus on their MIC, MBC, and phytochemical composition. Among all tested plant extracts, cinnamon emerged as the most potent antimicrobial agent, with its acetone extract demonstrating the lowest MIC (97.6 *µg/ml*) and MBC (195.2 *µg/ml*) values. This finding is in agreement with previous reports on the antimicrobial effects of cinnamon essential oil (7,20), although those studies primarily used essential oil preparations and did not explore organic solvent-based extracts in detail. The current results thus provide novel insights into the efficacy of cinnamon organic solvent extracts, particularly highlighting acetone as a highly effective extraction medium for anti-*Clostridial* compounds.

While some other spice extracts, such as those from clove, turmeric, and black pepper, exhibited moderate to strong inhibitory activity, they did not match the potency of cinnamon extracts. Notably, the petroleum ether extract of turmeric displayed a bacteriostatic effect rather than a bactericidal one, implying that although microbial growth was inhibited, complete killing was not achieved at the tested concentrations. These differences in antibacterial activity across solvents and spices can be largely attributed to the variability in phytochemical composition and their differential solubility in solvents of varying polarity.

The phytochemical screening conducted on all extracts revealed a diverse presence of terpenoids, flavonoids, glycosides, saponins, steroids, and alkaloids, with polar solvents like ethanol and methanol extracting the widest range of bioactive compounds. Interestingly, cinnamon extracts consistently exhibited a high presence of saponins, steroids, terpenoids, and alkaloids, and polar solvents such as acetone and ethanol proved especially effective in retrieving these bioactives. These findings were reinforced by statistical analyses. Two-way ANOVA and Tukey's HSD post-hoc tests confirmed that both spice type and solvent significantly affect MIC and MBC values ($p<0.00001$), with strong interaction effects, validating that certain spice-solvent combinations are uniquely effective.Although cinnamaldehyde's membrane-disruptive action has been extensively demonstrated in gram-positive organisms such as *Listeria monocytogenes*, similar mechanisms are plausible in *C. perfringens* due to its comparable phospholipid-rich membrane architecture. The MIC obtained for the cinnamon acetone extract in the present study (97.6 *µg/ml* ≈ 0.0976 *mg/ml*) is markedly lower than values reported for many other

spice-derived antimicrobials against *C. perfringens* (21) reported MICs of 1.25 *mg/ml* for thyme, 5.0 *mg/ml* for basil and marjoram, and 10 *mg/ml* for rosemary, peppermint, and anise, with anise showing only bacteriostatic activity (21). Similarly, (22) evaluated essential oils of aromatic plants and reported MIC values against *C. perfringens* ranging from 2.66 *mg/ml* to >76 *mg/ml*, depending on plant species and chemical composition (22). When compared with these findings, the cinnamon–acetone extract identified in the present work demonstrates a 10–1000-fold higher inhibitory potency, underscoring its exceptional efficacy relative to essential oil preparations commonly studied in literature.

The antibacterial activity observed in the present study is supported by well-established mechanistic actions of the bioactive phytochemicals present in the tested spices. The remarkable potency of the cinnamon acetone extract can be attributed primarily to cinnamaldehyde and other terpenoid/aldehyde constituents, which disrupt the bacterial cytoplasmic membrane, dissipate ion gradients such as K^+, collapse membrane potential, and cause leakage of intracellular components, ultimately leading to cell death (23).

Black pepper contains piperine, an alkaloid known to intercalate into bacterial membranes, increase membrane permeability, inhibit major efflux pumps (*e.g.*, MexAB-OprM) mechanisms that collectively reduce bacterial survival and virulence (24). Clove extracts are rich in eugenol, a phenylpropanoid that integrates into bacterial lipid bilayers, disrupts membrane integrity, increases permeability, and causes leakage of proteins and ions. Eugenol also inhibits ATPase activity and several bacterial virulence factors, thereby impairing metabolic energy production and reducing pathogenicity (25).

Star anise contains trans-anethole, which disrupts membrane potential, increases oxidative stress, induces intracellular accumulation of Reactive Oxygen Species (ROS), and causes protein/DNA damage in gram-positive bacteria. Anethole has also been shown to inhibit quorum sensing thereby acting as a potent anti-microbial agent (26). Turmeric owes its antibacterial activity primarily to curcumin, a polyphenolic compound that interacts with the bacterial membrane at both low and high concentrations. At low concentrations, curcumin binds to the lipid–water interface through hydrogen bonding with phospholipid headgroups, restricting water penetration and altering membrane hydration. At higher concentrations, curcumin penetrates deeper into the acyl chain region, causing packing defects, increasing membrane fluidity, and inducing phase separation. These membrane-altering effects disrupt membrane-bound proteins, impair transport processes, and contribute to growth inhibition (27).

Overall, these diverse mechanisms ranging from membrane disruption and oxidative stress induction to enzyme inhibition and interference with cell division help explain the differential antibacterial potency observed among the spice extracts tested in this study. It is therefore plausible that a combination of phytochemicals contributes to the observed activity, and further targeted chemical characterization and bioassay-guided fractionation are necessary to identify the exact bioactive constituents responsible for inhibiting *C. perfringens*. However, this potential synergistic interaction remains speculative, as the present study did not include fractionation or combination assays to verify synergism experimentally.

Another dimension to consider is the geographic and environmental variability in phytochemical content. Factors such as altitude, climate, and soil composition can influence the secondary metabolite profiles of plant materials, potentially affecting the concentration and composition of antimicrobial constituents in different regions. Hence, reproducibility of results across regions and harvests is an important aspect for future standardization.

In conclusion, the present study establishes cinnamon, particularly its acetone extract, as a highly effective natural antimicrobial agent against *C. perfringens*, supported by both quantitative MIC/MBC assays and qualitative phytochemical profiling. These findings provide a strong foundation for further pharmacological exploration of cinnamon and related spices, especially in the context of natural antibacterial agents targeting anaerobic pathogens. Future studies should aim to isolate and characterize the active compounds and assess their mechanisms of action individually and in synergy.

Conclusion

This study demonstrates that Indian spices, particularly cinnamon, exhibit strong antibacterial activity against *C. perfringens*, with the acetone extract of cinnamon showing the highest potency. The findings emphasize that both spice type and extraction solvent significantly influence antimicrobial efficacy, with polar and intermediate polarity solvents yielding more bioactive phytochemical constituents. These results support the potential of cinnamon derived compounds as natural antimicrobial agents, with possible applications in food preservation, surface sanitation, and formulation of plant-based disinfectants targeting anaerobic pathogens. Such applications could provide safer alternatives to synthetic preservatives and contribute to managing antimicrobial resistance.

Limitations and future scope

The present study was conducted using a single reference strain of *C. perfringens* (ATCC 13124), which limits the generalizability of the findings across diverse clinical isolates. The work relied entirely on crude solvent extracts, and no bioassay-guided fractionation or purified compound validation was performed, preventing precise identification of the active constituents responsible for antibacterial activity. Additionally, the study did not include cytotoxicity evaluation on mammalian cell lines, which is essential for determining therapeutic safety. Animal testing was not carried out in this phase, and therefore the *in vivo* efficacy, toxicity profile, pharmacokinetics, and stability of the extracts remain uncharacterized. Future work should incorporate *in vivo* animal models, detailed toxicity and biocompatibility assessments, quantitative phytochemical profiling, and mechanistic studies using isolated compounds. Investigation of synergistic interactions among cinnamaldehyde, eugenol, piperine, anethole, curcumin, and other phytochemicals will further strengthen the translational potential of these natural antimicrobials.

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Ethics approval and consent to participate

Not applicable.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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