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Possible mode of synergistic antimicrobial action of essential oil components cinnamaldehyde and eugenol in combination against foodborne bacteria *Listeria monocytogenes* and *Salmonella typhimurium*

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Abstract

Synergistic antimicrobial interaction is a promising strategy to overcome the challenges of microbial contamination in food. The aim of this study was to elucidate possible mode of synergistic antimicrobial interaction of essential oil components cinnamaldehyde and eugenol in combination against foodborne bacteria *Listeria monocytogenes* and *Salmonella typhimurium* using standard methods. At their individual effect, eugenol at 0.5×MIC concentration acted upon bacterial cell envelope leading to membrane damage and cytoplasm leakage whereas cinnamaldehyde at 0.5×MIC concentration failed to do so. But in combination, at their 0.5×MIC concentrations, eugenol allows cinnamaldehyde to enter inside the bacterial cells of studied bacterial pathogens with ease. Once they entered inside the cell, both eugenol and cinnamaldehyde simultaneously induced a significant disturbance in the cytoplasm of bacterial cells leading to significant release of cellular materials compared to their individual effects due to their synergistic interaction. The ultimate result is the loss of membrane integrity which caused a significant change in cellular morphology and subsequently led to cell lysis and cell death. The results provide evidence that eugenol/cinnamaldehyde blend at sufficiently low concentration may serve as a more potent natural alternative to synthetic antimicrobial food preservatives in food industry. This study can provide an interesting platform in the near future for this area of research. To the best of our knowledge this paper is the first to study possible mode of synergistic antimicrobial interactions of essential oil components in combination against foodborne Gram-positive and Gram-negative bacteria.

Keywords: Food Safety; Essential Oil Components; Natural Antimicrobials; Synergistic Interactions; Mode of Antimicrobial Action; Natural Food Preservatives

1. Introduction

Food spoilage and contamination caused by microorganisms is a worldwide food safety challenge for both consumers and the food industry. Therefore, preventive measures are essential to reduce food loss and protect consumers from foodborne illnesses (Gustavsson et al. 2011; Kirk et al. 2017). The use of synthetic antimicrobial compounds as food preservatives has raised consumers' concerns since they have accumulated evidence that they could be toxic and carcinogenic (Anand and Sati 2013; Singh et al. 2019). This problem demands that a new effort should be made to seek safe and effective novel natural antimicrobials from other sources especially from plant origin (Burt 2004; Hyldgaard et al. 2012). Essential oils are generally regarded as safe (GRAS) by the U.S. Food and Drug Administration (FDA 2020). Efficacy of essential oils and some of their active components as potent antimicrobials against a number of pathogens and food contaminants has been reported by several workers (Gutierrez et al. 2008; Soliman et al. 2017; Mo &Os 2017; Vasireddy et al. 2018), suggesting their possible application in food industry (Burt 2004; Chouhan et al. 2017). But, the main obstacle for using essential oils and their active components as food grade preservatives is that they are not potent

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enough as single components, and their high concentration is required to get desired activities which may cause negative organoleptic impact in foods (Hyldgaard et al. 2012). Exploiting synergies between several bioactive compounds and detailed understanding about their mode of interactions have been suggested as a possible solution to this problem (Burt 2004; Hyldgaard et al. 2012). Such knowledge could contribute to design of new and more potent antimicrobial blend effective at sufficiently low concentration and this understanding is also imperative to design strategies to eradicate foodborne microbes without compromising the organoleptic properties of foods (Hyldgaard et al. 2012; Chouhan et al. 2017; Angane et al. 2022). Our previous study revealed that cinnamon oil and clove oil in combination exhibited synergistic antibacterial interaction against foodborne bacteria *Listeria monocytogenes* and *Salmonella typhimurium* (Purkait et al. 2020). Chemical analysis revealed that bioactive compounds responsible for synergistic antibacterial interaction against the studied bacterial pathogens are cinnamaldehyde from cinnamon oil and eugenol from clove oil (Purkait et al. 2021). In the present investigation, an attempt has therefore been made to elucidate possible mode behind the synergistic antimicrobial interaction of these two active essential oil components cinnamaldehyde and eugenol against foodborne bacteria *L. monocytogenes and S. typhimurium* with a view to contribute more knowledge in the relevant field for their plausible application in food industry as safe and effective novel natural antimicrobial blend.

2. Materials and Methods

2.1. Collection and maintenance of test microbes

The bacterial strains used in this study were *Listeria monocytogenes* (MTCC 657) and *Salmonella typhimurium* (MTCC 3224) as indicator strains of Gram-positive and Gram-negative bacteria respectively. These strains were procured from the Institute of Microbial Technology, Chandigarh, India. For standardization, test bacterial strains were incubated at respective temperature (30°C for *L. monocytogenes* and 37°C for *S. typhimurium*) in nutrient broth for 3-6h to attain a turbidity of 0.5 McFarland Standard. The inoculum size was adjusted to 5×10^5CFU/ml following CLSI guidelines (CLSI 2005).

2.2. Chemicals

The essential oil components cinnamaldehyde and eugenol used in the present investigation were isolated, identified and chemically characterized from cinnamon oil and clove oil respectively (Purkait et al. 2021). Other chemicals were procured from *E. Merck* KG, Darmstadt, *Germany*, unless stated otherwise.

2.3. Elucidation of mode of synergistic antimicrobial interaction

2.3.1. Effects on cell viability

Kill-kinetics assay

Effects of test compounds cinnamaldehyde and eugenol alone and in combination against cell viability of studied bacterial pathogens *L. monocytogenes* (MTCC657) and *S. typhimurium* (MTCC 3224) were evaluated by kill-kinetics assay following the method of Zhou et al. (2016). Briefly, 100µl of bacterial inoculum (5×10^5 CFU/ml), 100 µl of test compounds individually at their 1×MIC concentration and in combination (1:1) at their 0.5×MIC concentrations were taken in test tubes each containing 10ml of nutrient broth. The tubes were then incubated at respective temperature (30°C for *L. monocytogenes;* 37°C for *S. typhimurium)* for 24h. 500µl sample was collected from culture tubes at different time intervals (0-, 3-, 6-, 12- and 24-h of incubation). Collected samples were then diluted serially with nutrient broth and 100µl of diluted sample was spread evenly on fresh nutrient agar media and incubated for 24h at respective temperature (30°C/37°C) for viable cell count ($\log_{10}$ CFU/ml). Samples without test compounds served as negative control. Kill-kinetics curves [$\log_{10}$ CFU/ml Vs. Time (h)] were plotted to know the effects of test compounds alone and in combination on rate and extent of killing of studied bacterial pathogens. Each experiment was repeated thrice.

2.3.2. Effects on cell membrane integrity

Measurement of release of 260-nm absorbing cellular materials

To determine the effects of test compounds alone and in combination on cell membrane integrity of studied bacterial pathogens, release of 260-nm absorbing materials (DNA and RNA) of the cells was measured following the method described by Chen and Cooper (2002). Briefly, bacterial suspension was prepared from overnight culture of test bacterial strains. Cells were separated from medium by centrifugation at 1000×g for 10 min, washed twice in phosphate buffered saline (pH 7.4), and re-suspended in the same buffer. 100 µl of different concentrations of test compounds alone at their 1×MIC concentration and in combination (1:1) at their 0.5×MIC concentrations were added to 100 µl of cell suspension (5×10^5 CFU/ml) of studied bacterial strains. Cell suspension without test compounds served as negative control. Samples were incubated at respective temperature (30°C/37°C) for 24h and filtered. The absorbance of the supernatant was then measured at 260 nm. All experiments were repeated thrice.

Measurement of leakage of cellular proteins

Effect of test compounds on cell membrane integrity was also evaluated by determining their individual and combined effects on release of cellular proteins in the supernatant of bacterial cells following Bradford's method (1976). In brief, 100µl of an overnight culture of bacterial cells (5×10^5 CFU/ml) was treated with 100 µl of test compounds alone at their 1×MIC concentration and in combination (1:1) at their 0.5×MIC concentrations and incubated for 24 h at respective temperature (30°C/37°C). 100µl of sample was then mixed with 400µl of Bradford reagent and incubated for further 10 min at room temperature. After incubation, absorbance of the samples was measured at 595 nm using a spectrophotometer. Control samples were treated similarly without test compounds and served as negative control. Bovine serum albumin (BSA) was used as a reference standard protein. Each experiment was repeated thrice.

2.3.3. Effects on cell membrane permeability

Measurement of leakage of extracellular potassium ions

To determine the effects of test compounds alone and in combination on cell membrane permeability of studied bacterial pathogens, the release of extracellular potassium ions of studied bacterial pathogens before and after treatment of test compounds was measured following the method of Lee et al. (2002) with slight modifications. Briefly, 100 µl of overnight culture of bacterial cells (5×10^5 CFU/ml) were treated with 100 µl of test compounds alone at their 1×MIC concentration and in combination (1:1) at their 0.5×MIC concentrations and incubated for 2h at respective temperature (30°C/37°C). Incubated samples were collected at 30 min, 60 min, 90 min and 120 min intervals. The release of potassium ion of samples at each time interval was measured using the Kalium/Potassium kit (Quantofix, GmbH, Wiesbaden, Germany). Control samples were treated similarly without test compounds. Results were expressed as the amount of extracellular free potassium ion (K^+ ion) (µM) released at each time interval of incubation period. Each experiment was repeated thrice.

2.3.4. Effects on cellular morphology

Scanning Electron Microscopy (SEM) analysis

To evaluate the effects of test compounds alone and in combination on cellular morphology of studied bacterial pathogens, scanning electron microscopy (SEM) analysis following the method of Benli et al. (2008) was performed. Briefly, 100µl of an overnight culture of bacterial cells (5×10^5 CFU/ml) were treated with test compounds individually at their 1 ×MIC concentration and in combination (1:1) at their 0.5×MIC concentrations. Samples were then incubated at respective temperature (30°C/37°C) for 24h, centrifuged at 10000×g for 10 min and filtered. The pellet was washed slowly with 50mM phosphate buffer solution (pH 7.2), mounted over glass slides and fixed with 2.5% glutaraldehyde followed by dehydration using various concentrations (50% -100%) of ethanol. Finally, ethanol was replaced by t-butanol and incubated at room temperature for 2h. Control samples were treated similarly without test compounds. The samples were then subjected to gold sputtering (sputter coating k500, Emitech) for 120s and observed for possible changes in cellular morphology of treated cells compared to their controls by scanning electron microscopy (SEM) (S-4100; Hitachi).

2.4. Statistical Analysis

Statistical analysis was performed using SPSS software: Version 18.0 with the level of significance set at $p < 0.05$.

3. Results and Discussion

Various modes of antimicrobial action of essential oils and their bioactive compounds have been proposed by several workers. It has been reported that essential oils have the ability to disrupt cell membrane integrity and membrane permeability leading to cellular damage and subsequent release of intracellular materials (Cox et al. 2000; Carson et al. 2002; Burt 2004; Rota et al. 2004). It has also been reported that antimicrobial activity of essential oils is dependent on their composition, configuration, amount and their possible interactions (Lis-Balchin et al. 1998). Considering these, to achieve our goal, in the present investigation we evaluated the effects of test compounds eugenol and cinnamaldehyde alone at their 1 ×MIC concentration and in combination at their 0.5×MIC concentrations on cell viability, cell membrane integrity, cell membrane permeability and cellular morphology against both Gram-positive and Gram-negative foodborne bacterial pathogens. The MICs of eugenol against *L. monocytogenes* (MTCC 657) and *S. typhimurium* (MTCC 3224) were found to be 70.74 ± 2.16 µg/ml and 85.43±3.54 µg/ml respectively whereas these values for cinnamaldehyde against *L. monocytogenes* (MTCC 657) and *S. typhimurium* (MTCC 3224) were found to be 75.18±2.16 µg/ml and 114.63±3.44 µg/ml respectively in our previous experiment (Purkait et al. 2021).

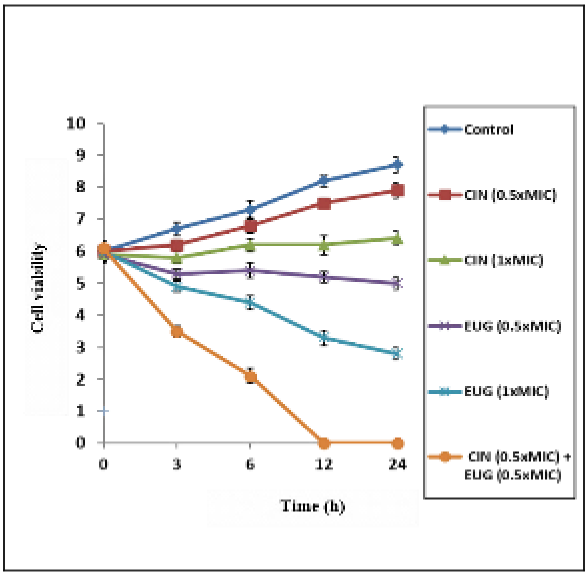

Figure 1 a Effect of cinnamaldehyde and eugenol alone and in combination on cell viability (Log_{10} CFU/ml) against *L. monocytogenes* (MTCC 657). Error bars indicate standard deviation; CIN: Cinnamaldehyde; EUG: Eugenol

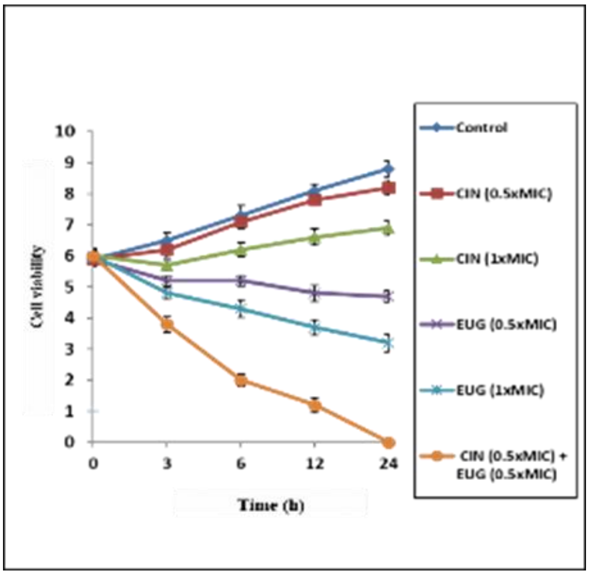

Figure 1b Effect of cinnamaldehyde and eugenol alone and in combination on cell viability (Log_{10} CFU/ml) against *S. typhimurium* (MTCC 3224). Error bars indicate standard deviation; CIN: Cinnamaldehyde; EUG: Eugenol

Effects on cell viability was evaluated by performing kill-kinetics assay (Schaper et al. 2005). From kill-kinetics curves of studied bacterial pathogens *L. monocytogenes* (Figure 1a) and *S. typhimurium* (Figure 1b), it was observed that cinnamaldehyde at its individual effect at 0.5×MIC concentration failed to reduce significantly the viable cell count of both the studied bacterial pathogens compared to respective controls throughout the incubation period. However, at 1×MIC concentration, the reduction in viable cell count by cinnamaldehyde against both the studied bacterial pathogens was found to be significant ($p < 0.05$) from 3h onward. On the other hand, eugenol at its individual effect at 0.5×MIC concentration had significant effect ($p < 0.05$) on reduction in viable cells against both the studied bacteria from 3h onward. But in combination with cinnamaldehyde at their 0.5×MIC concentrations, a reduction in colony count by >3log_{10} CFU/ml was observed against both the studied bacterial pathogens compared to controls from 3h onwards and a complete reduction in cell viability by test compounds in combination at their 0.5×MIC concentrations was observed at 12h against *L. monocytogenes* (Figure 1a) and at 24h against *S. typhimurium* (Figure 1b) suggesting their time-dependent strain-specific antibacterial activity against studied bacterial pathogens. They were also found to be more effective both at their individual and combined effects against *L. monocytogenes* compared to *S. typhimurium* which was most likely to be due to differences in the cell membrane composition of studied bacterial pathogens (Gutierrez et al. 2008) as well as chemical composition of test bioactive compounds (Sikkema et al. 1995; Gill and Holley 2004).

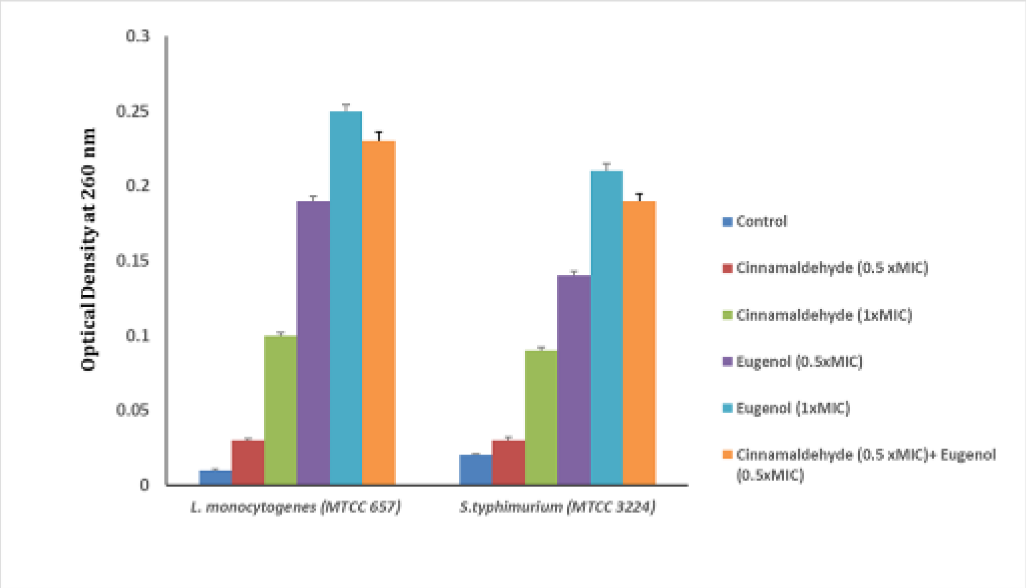

Figure 2 Effect of cinnamaldehyde and eugenol alone and in combination on release of 260-nm absorbing materials of *L. monocytogenes*(MTCC 657) and *S. typhimurium*(MTCC 3224)

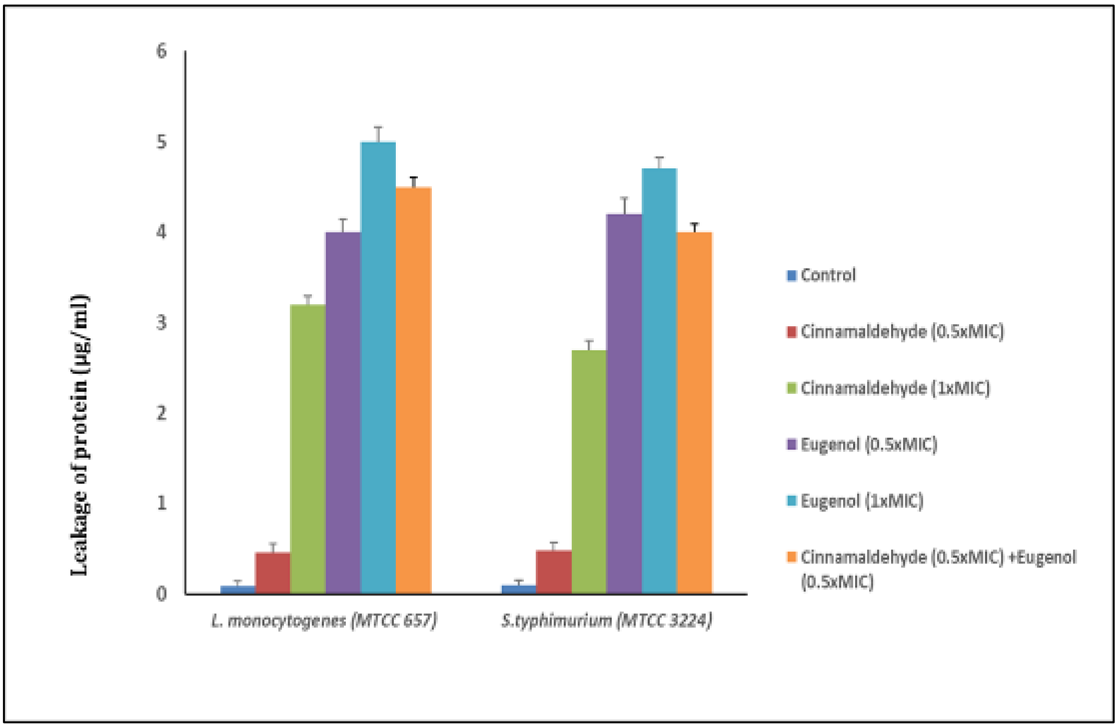

Figure 3 Effect of cinnamaldehyde and eugenol alone and in combination on leakage of cellular proteins of *L. monocytogenes* (MTCC 657) and *S. typhimurium* (MTCC 3224)

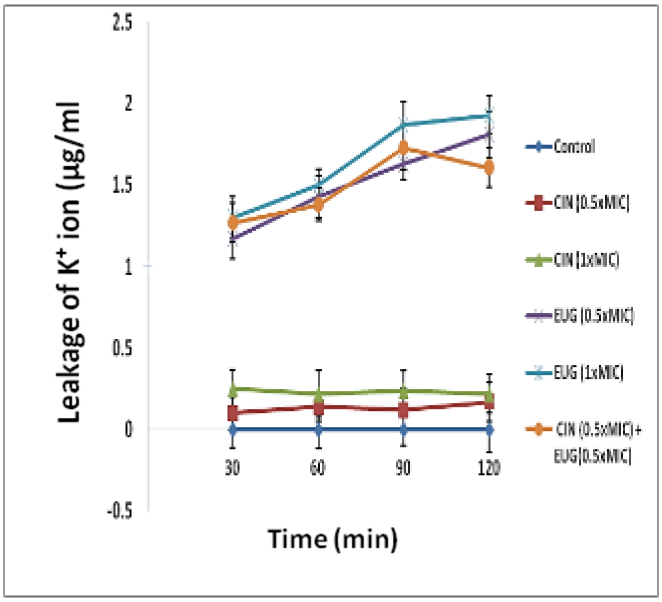

Figure 4a Effects of cinnamaldehyde and eugenol alone and in combination on leakage of potassium ions of *L. monocytogenes* (MTCC 657)**;** CIN: Cinnamaldehyde; EUG: Eugenol

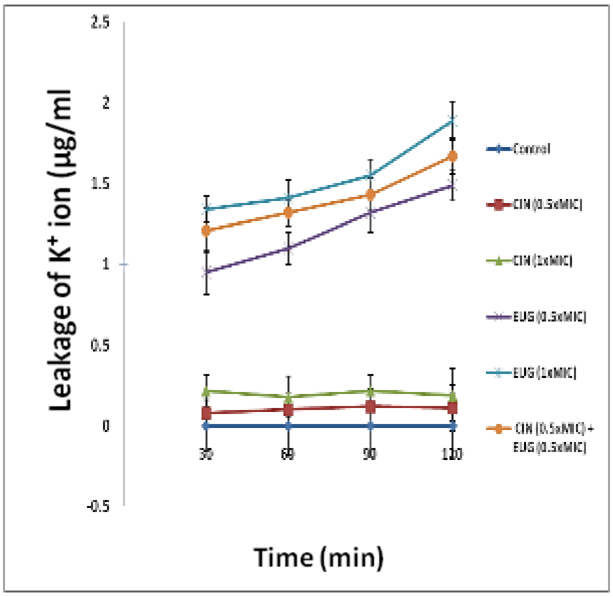

Figure 4b Effects of cinnamaldehyde and eugenol alone and in combination on leakage of potassium ionsof *S. typhimurium* (MTCC 3224); CIN: Cinnamaldehyde; EUG: Eugenol

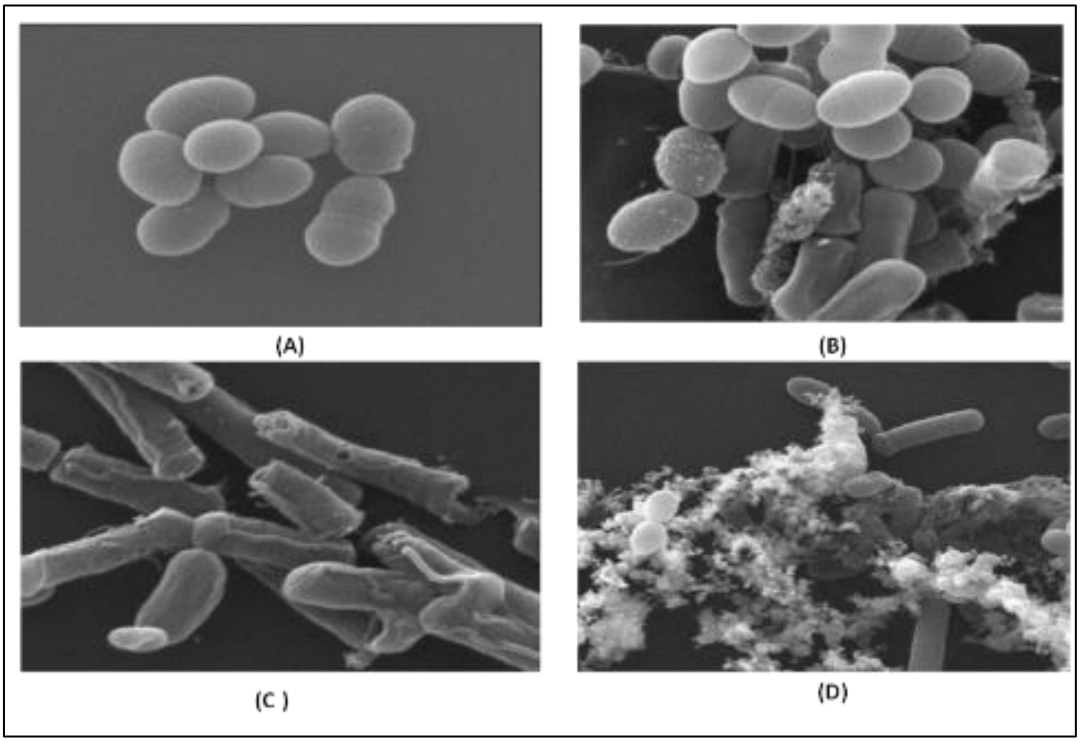

Figure 5 (A-D) SEM images of cellular morphology of *L. monocytogenes* (MTCC 657): (A) control; (B) cinnamaldehyde (1×MIC) treated; (C) eugenol (1×MIC) treated and (D)cinnamaldehyde (0.5 ×MIC) + eugenol (0.5 ×MIC) treated

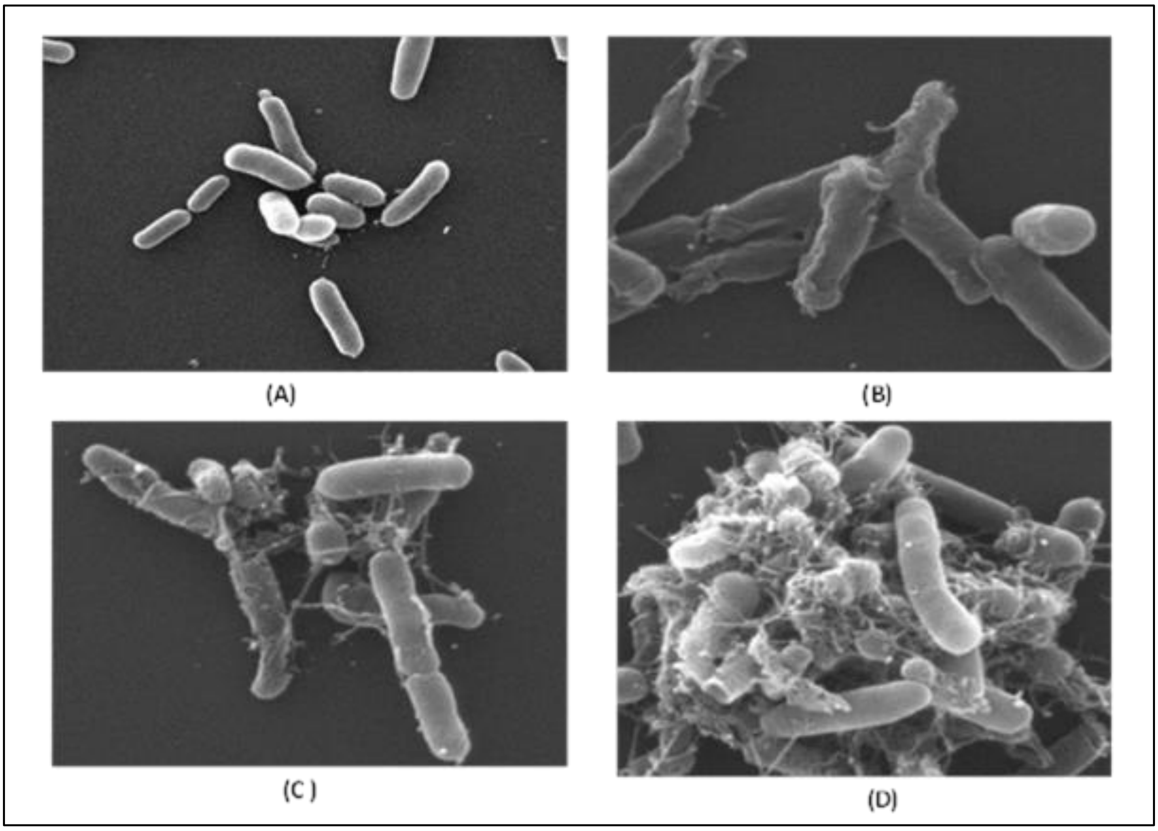

Figure 6 (A-D) SEM images of cellular morphology of *S. typhimurium* (MTCC 3224) : (A) control; (B) cinnamaldehyde (1×MIC) treated; (C) eugenol (1×MIC) treated and (D) cinnamaldehyde (0.5 ×MIC) + eugenol (0.5 ×MIC) treated

Next, we evaluated the effects of test compounds alone and in combination on release of 260-nm absorbing materials (DNA, RNA) (Figure 2) and leakage of cellular proteins (Figure 3) to know the effects of test compounds on cell membrane integrity of studied bacterial pathogens (Carson et al. 2002). It was observed that the test bioactive compounds at their individual effect exhibited varying degrees of concentration-dependent activity on release of 260-nm absorbing materials and cellular proteins against both the studied bacterial pathogens. Eugenol by itself at low concentration (0.5×MIC) increased the release of both 260-nm absorbing materials and cellular proteins significantly ($p< 0.05$) against the studied bacterial pathogens compared to controls whereas cinnamaldehyde by itself at low concentration (0.5×MIC) failed to do so. In combination, at their 0.5×MIC concentrations, the release of 260-nm absorbing materials as well as cellular proteins of studied bacterial pathogens were found to be significantly greater ($p<0.05$) compared to their individual effects (Figure 2 and Figure 3) suggesting their higher effectiveness against the studied bacterial pathogens in combination at low concentration compared to their individual effects.

Relevant literature reveals that the internal environment of bacterial cells is generally rich in potassium (K^+) ions and their presence in the extracellular medium is an indication of cytoplasmic membrane damage (Newman and Cragg 2012; Carson et al. 2002; O'Bryan et al. 2015). Therefore, to observe the effects of test bioactive compounds on cell membrane permeability of studied bacterial pathogens, their individual and combined effects on release of extracellular potassium ions against the studied bacterial pathogens were evaluated and the obtained results are shown in Figure 4a (*L. monocytogenes)* and Figure 4b (*S. typhimurium)*. It was observed that cinnamaldehyde at its individual effect at 0.5×MIC concentration failed to release significantly the extracellular potassium ions of studied bacterial pathogens compared to its control values and even at 1×MIC concentration, it did not show any significant alteration in release of extra cellular potassium ion compared to its 0.5×MIC concentration. But, eugenol even at 0.5×MIC concentration at its individual effect caused a significant release ($p< 0.05$) in extracellular potassium ions of both the studied bacterial pathogens compared to their control values, and this was further increased at its 1×MIC concentration. However, in combination with cinnamaldehyde at their 0.5×MIC concentrations, the release of extracellular potassium ions was increased significantly ($p< 0.05$) in both the studied bacterial pathogens compared to their control values. The findings indicate that eugenol at its individual effect has the ability to increase membrane permeability at low concentration of studied bacterial pathogens but cinnamaldehyde at its individual effect even at higher concentration (1×MIC) failed to do so. In combination at lower concentration (0.5×MIC), the increased release of extracellular potassium ions of studied bacterial pathogens might be due to their synergistic interactions on bacterial cytoplasm.

The findings of higher antibacterial efficacy of eugenol compared to cinnamaldehyde at their individual effect against the studied bacterial pathogens obtained in our present investigation are in corroboration with the findings of other workers where eugenol showed higher antibacterial efficacy than cinnamaldehyde against a number of microbes (Helander et al. 1997; 1998; Gill & Holley 2004; Nazzaro 2013). Possible reasons behind the differences in mode of antibacterial action between eugenol and cinnamaldehyde at their individual effect against the studied bacterial pathogens observed in our present investigation is not clear right now. Relevant literature reveals that eugenol is a lipophilic molecule which can actively penetrate the cell membrane lipid bilayer (Atsumi et al. 2001). Besides, eugenol has a phenolic group, which has the ability to destroy the cell membrane (Sikkema et al. 1995). Furthermore, such a phenolic group is actually widely utilized in the extraction of lipopolysaccharide (LPS) from bacteria (Helander 1983). Thus, it can be presumed that the strong membrane damaging effect of eugenol at its individual effect at lower concentration on the studied bacterial pathogens may be due to its lipophilic nature and it contains phenolic group in its molecular structure. On the other hand, cinnamaldehyde by itself at its lower concentration was found to have no effect on the cell membrane of studied bacterial pathogens. This may be due to the fact that cinnamaldehyde does not possess any –OH group which has the ability to damage membrane function at low concentration (Gill & Holley 2004). Its antimicrobial action by destabilizing the outer membrane at higher concentration was also observed by other workers against *E. coli*, and *S. typhimurium* (Helandel et al. 1998; Nazzaro et al. 2013). The inability of cinnamaldehyde to access the bacterial cells at low concentration at its individual effect, may be due lack of aromatic hydroxyl group (-OH) in its chemical structure for which at low concentration it is pumped out from the periplasm of bacterial pathogens at a rate exceeding its penetration rate (Nikaido 1994).

Next, scanning electron microscopy (SEM) analysis was performed to characterize the surface features and also to evaluate the morphological changes of cell membrane of studied bacterial pathogens before and after treatment with test bioactive compounds alone and in combination with a view to support our previous findings. The images of scanning electron microscopy analysis of studied bacterial pathogens are shown in Figure 5(A-D) for *L.monocytogenes* and Figure 6(A-D) for *S. typhimurium*. It was observed that the cellular morphology of control groups of studied bacterial pathogens exhibited a normal shape and smooth surface [Figure 5 (A) and Figure 6 (A)]. After treatment with cinnamaldehyde alone at 1×MIC concentration, the overall morphology of both the studied bacterial cells showed a negligible change compared to the cellular images of control cells (Figure 5B and Figure6B). But the cellular morphology of studied bacterial pathogens was found to be moderately damaged by eugenol at 1×MIC concentration at its individual effect (Figure 5C and Figure 6C). In combination of test bioactive compounds, at their 0.5 × MIC concentrations, there was a significant alteration in cellular morphology of both the studied bacterial pathogens (Figure5D and Figure 6D) compared to their control images (Figure 5A and Figure 6A) was observed. Scanning electron microscopy analysis results indicate that at their individual effect, they showed strain-specific little to moderate alternations in cellular morphology of studied bacterial pathogens and eugenol was found to be more potent antibacterial agent than cinnamaldehyde against both the studied bacterial pathogens. In combination at their 0.5×MIC concentrations, they could destroy the structure of studied bacterial pathogens by inducing serious alterations in the intracellular organization, and lead to the outflow of cytoplasmic components which may be due to their synergistic antimicrobial interactions in combination.

4. Conclusions

From the foregoing findings it was observed that mode of antibacterial action of cinnamaldehyde and eugenol at their individual effect against the studied bacterial pathogen is different. Eugenol at its individual effect at lower concentration, acted upon bacterial cell envelope of both the studied bacterial pathogens leading to membrane damage and cytoplasm leakage whereas cinnamaldehyde at its individual effect at same concentration failed to damage bacterial cell membrane as well as cytoplasm leakage which may be due to differences in their chemical nature. In combination, at their lower concentrations, eugenol due to its lipophilic nature with a hydroxyl group in its chemical structure, increased membrane permeability of both the studied bacterial pathogens and allows cinnamaldehyde to enter at its lower concentration inside the bacterial cells with ease. Once they entered inside the cell, both eugenol and cinnamaldehyde simultaneously possibly due to their synergistic interactions induced a significant disturbance in the cytoplasm of bacterial cells leading to significant release of cytoplasmic materials. At the end, the loss of membrane integrity caused a significant change in cellular morphology which subsequently led to cell lysis and cell death. These promising findings may have significant impact in food industries for the development of a more potent and best suited promising natural alternative to synthetic antimicrobial food preservatives. The combination of these two active essential oil components at lower concentration may also be helpful in minimizing the undesirable impact of essential oils on sensory properties of food as well as to overcome the problem of multidrug-resistant microorganisms. This promising report may serve as a footstep on these important aspects.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

The authors have no relevant financial or non-financial interests to disclose.

Statement of informed consent

This article does not contain any studies with human participants or animals performed by any of the authors

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Authors Contributions

Rabi Ranjan Chattopadhyay contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shilpa Purkait. The first draft of the manuscript was written by Shilpa Purkait. All authors commented on the previous version of the manuscript. All authors read and approved the final manuscript.

References

[1] Anand SP, Sati N (2013) Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. Int J Pharm Sci 4(7): 2496-2501.

[2] Angane M, Swift S, Huang K, Butts CA, Quek SY (2022) Essential oils and their major components: An updated review on antimicrobial activities, mechanism of action and their potential application in the food industry. *Foods 11*:464.

[3] Atsumi T, Iwakura I, Fujisawa S, Ueha T (2001) Reactive oxygen species generation and photo-cytotoxicity of eugenol in solutions of various pH.*Biomaterials* 22(12): 1459-1466.

[4] Benli M, Yigit N, Geven F, Guney K, Bingol U (2008) Antimicrobial activity of endemic *Crataegus tanacetifolia* (Lam.) Pers and observation of the inhibition effect on bacterial cells. Cell Biochemistry and Function: Cellular Biochemistry and Its Modulation by Active Agents or Disease26(8): 844-851.

[5] Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem**72**(1-2): 248-254.

[6] Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Micro 94(3): 223-253.

[7] Carson CF, Mee BJ, Riley TV (2002) Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. Antimicrob Agents Chemother46(6): 1914-1920.

[8] Chen CZ, Cooper SL (2002) Interactions between dendrimer biocides and bacterial membranes. Biomaterials 23(16): 3359-3368.

[9] Chouhan S, Sharma K, Guleria S (2017) Antimicrobial activity of some essential oils-present status and future perspectives. Medicines (Basel)3: 58.

[10] CLSI. 2005. Performance standards for antimicrobial susceptibility testing: fifteenth informational supplement, CLSI document M100–S15. CLSI Weyne.

[11] Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, Wyllie SG (2000) The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J App Micro 88(1): 170-175.

[12] GillA O, Holley RA (2004) Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. Appl Env Micro 70:5750–5755.

[13] Gustavsson J, Cederberg C, Sonesson U, Otterdijk R, Maybeck A (2011) *Global Food Losses and Food Waste: Extent, Causes, and Prevention*. Düsseldorf: FAO.

[14] Gutierrez J, Barry-Ryan C, Bourke P (2008) The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int J Food Microbiol124(1): 91-97.

[15] Helander IM, Alakomi HL, Latva K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LGM, von Wright A (1998) Characterization of the action of selected essential oil components on Gram-negative bacteria. J Agric Food Chem46:3590–3595.

[16] Helander IM, Alakomi HL, Latva-Kala K, Koski P (1997) Polyethyleneimine is an effective permeabilizer of Gram-negative bacteria. Microbiology143:3193–3199.

[17] Helander I, Hakalehto E, Ahvenainen J, Haikara A (1983) Characterization of lipopolysaccharides of *Pectinatuscerevisiophilus*. FEMS Micro Let 18:223-226.

[18] Hyldgaard M, Mygind T, Meyer RL (2012) Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Micro3:12.

[19] Kirk M D, Angulo FJ, Havelaar A H, Black RE (2017) Diarrhoeal disease in children due to contaminated food. Bull World Health Org 95 :233–234.

[20] Lee JY, Kim YS, Shin DH (2002) Antimicrobial synergistic effect of linolenic acid and monoglyceride against *Bacillus cereus* and *Staphylococcus aureus*. J Agric Food Chem 50: 2193–2199.

[21] Lis Balchin M, Deans SG, Eaglesham E (1998) Relationship between bioactivity and chemical composition of commercial essential oils. Flav Frag J 13(2): 98-104.

[22] Mo T, Os A (2017) Plant Essential Oil: An Alternative to Emerging Multidrug Resistant Pathogens. J Micro Exp5:1–10.

[23] Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V (2013) Effect of essential oils on pathogenic bacteria. Pharmaceuticals (Basel) 6(12):1451-74.

[24] Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75(3): 311-335.

[25] Nikaido H (1994) Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science 264 (5157): 382-388.

[26] O'Bryan CA, Pendleton SJ, Crandall PG, Ricke SC (2015) Potential of plant essential oils and their components in animal agriculture–*in vitro* studies on antibacterial mode of action. FrontVet Sc 2: 35.

[27] Purkait S, Bhattacharya A, Bag A, Chattopadhyay RR (2020). Synergistic antibacterial, antifungal and antioxidant efficacy of cinnamon and clove essential oils in combination. Arch Micro 202(6):1439-1448.

[28] Purkait S, Bhattacharya A, Bag A, Chattopadhyay RR (2021) TLC bioautography–guided isolation of essential oil components of cinnamon and clove and assessment of their antimicrobial and antioxidant potential in combination. Environ Sc Poll Res28(1):1131-1140.

[29] Rota C, Carraminana JJ, Burillo J, Herrera A (2004) *In vitro* antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens. J Food Protect 67(6):1252-1256.

[30] Schaper KJ, Schubert S, Dalhoff A (2005) Kinetics and quantification of antibacterial effects of beta-lactams, macrolides, and quinolones against gram-positive and gram-negative RTI pathogens. Infection 33(2):3-14.

[31] SikkemaJ, de Bont JA, Poolman B (1995) Mechanisms of membrane toxicity of hydrocarbons. Micro Rev 59(2):201-222.

[32] Singh B, Singh JP, Kaur A, Singh N (2019) Antimicrobial potential of pomegranate peel: A review. Inter J Food Sc Tech 54:959–965.

[33] Soliman SSM, Alsaadi AI, Youssef EG, Khitrov G, Noreddin AM, Husseiny MI, Ibrahim AS (2017) Calli Essential Oils Synergize with Lawsone against Multidrug Resistant Pathogens. Molecules 22(12):2223.

[34] U.S. Food and Drug Administration (2020) Code of Federal Regulations—Title 21—Food and drugs. U.S. Food and Drug Administration, Silver Spring, MD.

[35] Vasireddy L, Bingle LEH, Davies MS (2018) Antimicrobial activity of essential oils against multidrug-resistant clinical isolates of the *Burkholderiacepacia* complex. *PLoSONE*13:e0201835. doi: 10.1371/journal.pone.0201835.

[36] Zhou D, Wang D, Yang L, Liu Z, Zhang Y (2016) A modified and improved assay based on microbial test system (MTS) to evaluate antioxidant activity. Food Anal 9(4): 895-904.