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Antimicrobial Activity of Essential Oils Against Microbes Associated with Street Food

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Abstract
This review aims to evaluate the effectiveness of five essential oils (EOs), namely clove, lemongrass, mint, oregano, and black cardamom, at different concentrations, in fighting microorganisms that were isolated from selected food samples obtained from local street food vendors. The microorganisms isolated from the food samples were *Enterobacter* sp., *Pseudomonas* sp., *Proteus* sp., *Acinetobacter* sp., *Moraxella* sp., *Escherichia* sp. (gram-negative bacteria), and *Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp., *Lactobacillus* sp., *Micrococcus* sp. (gram-positive bacteria). The antimicrobial effects of these oils were tested, and the results were analyzed using one-way analysis of variance (ANOVA) with a significance level of $p<0.001$. The tests were carried out using different concentrations of 20%, 40%, 60%, 80%, and 100% (v/v) against isolates obtained from raw and street food. It was found that clove oil had the highest effect, followed by mint oil, black cardamom oil, and oregano oil. However, lemongrass oil showed the least antimicrobial effect. The results indicate that clove, mint, and black cardamom EOs are highly effective in controlling pathogenic bacteria. These oils can be used to prevent bacterial growth, extend shelf life, and increase the safety of street food.

Keywords: Natural Biocides, Antimicrobial Activity, Street and Raw food, Essential Oils

Introduction

Bacteria always exist right through food production and infect food products in various ways, at the farming stage, by workers, fecal contamination by animals and insects. They are also contaminated by post-harvest sources like handling by workers, processing equipment and infected from other food. These bacteria cause two major problems to food suppliers food borne sickness and monetary loss related to food loss because of food contamination [1]. The function of food additives is to reduce or inactivate the bacteria present in Street and packed foods [2]. The excess utilization of chemical preservatives reduces the quality of food due to their probable toxicity, as they possess toxicological problems like microbiological spoilage which changes the food pattern and growth of pathogens. There is also a growing demand by food manufacturers to replace chemical-based preservatives from food products to reduce their side effects [3]. This makes food industries seek “green” and “clean” logos for their product market outreach. Consumers and food manufacturers are being advised by WHO to reduce salt levels in processed food due to heart-related issues arising from high salt intake, but salt is a common preservative in most processed food. Consumers are aware of the use of processed food having no chemical preservatives [4]. Today’s trend is making use of natural additives or biocides rather than chemical preservatives.

"Biocides," which are natural plant components, have the ability to preserve food in various ways without harming the environment or people [5].

Plant-based extracts are fragrant oily liquid extracts from plants, mostly spices and herbs [6]. EOs have anti-microbial activity against a large variety of pathogens [7]. EOs with high vapor pressure have anti-bacterial potential against microorganisms (through gas and liquid phase) has been reported under *in vitro* conditions [8]. The mixture of EOs from thyme with sage and oregano with marjoram are active against *Listeria monocytogens* and *Escherichia coli*, they were found effective only when applied individually [9]. EOs and extracts from these plants - *Ocimum basilicum, Coriandrum sativum, Citrus limon, Cuminum cyminum, Anethum graveolens, Zingiber officinale, Laurus nobilis, Cymbopogon, Origanum majorana, Myristica fragrans, Salvia rosmarinus, Salvia officinalis, Satureja hortensis, Melaleuca alternifolia* show medium to high activity against foodborne pathogens [10]. Eugenol is the component with anti-bacterial activity by clove-bud (*Eugenia caryophyllata*) oil, comprising around 70-90% oil. Oregano oil is a common source of carvacrol, which makes up 60-70% of the oil [11].

The present study aims to show the antimicrobial effect of EOs on food borne bacteria. EOs, which are plant-based biocides, can be used as preservatives to reduce the use and impact of chemical preservatives, thereby enhancing food quality. The investigation conducted to determine the effect of essential oils (EOs) on microorganisms present in street food is as follows: (i) Evaluation of the incidences of microbes associated with street food responsible for food poisoning. (ii) Screening and bio-efficacy of plant-based biocides as essential oils (EOs) against test bacteria. (iii) To initiate a piece of advice for effective EOs that can be utilized as bio-additives for food.

Methods and Materials

Collection of street food samples for study Food samples were collected from the market for microbiological analysis as raw food (orange, pomegranate, broccoli, banana, grapes, raw milk, potato, capsicum, cucumber, tomato, sugarcane) and street food (tomato ketchup, green chutney, red chutney, orange juice, pani puri, pickle, curd, chips, chach, mixed juice, papaya shake, pineapple juice). A total of 24 raw and street food samples were aseptically collected from local vendors using sterile sampling tools and placed in labelled sterile containers.

Isolation and identification of bacterial isolate Microbiological analysis of the food samples for determining bacteria responsible for food-borne infection. Isolation of microorganisms were done as follows: we prepared dilutions ranging from 10^{-1} to 10^{-9}. To make the 10^{-1} dilution, we dispensed 1 gm of the sample in 9 mL of sterile water, mixed it well, and let it settle. Then, we homogenized 1mL of this mixture in another test tube with 9 mL of sterile water, resulting in a 10-fold dilution. We used this dilution to obtain the other dilutions up to 10^{-9}. Chromogenic broth and agar supplemented with sodium azide for gram-positive bacteria [12,13] and for gram-negative bacteria supplemented with phenol red and brilliant green [14] were prepared. Agar plates were inoculated with diluted food samples using the streaking technique and incubated at 37° C for 24 hours. Qualitative and quantitative analysis of standard plate count (SPC) were done to determine the diversity and number of bacteria. The bacterial isolates were identified by following the guidelines provided in Bergey's Manual of Systematic Bacteriology [15]. Morphological analysis was done as follows: morphology of colony: appearance, shape of colony, colours, elevation, margin. Microscopic analysis was done by gram staining. Biochemical analysis was done based on biochemical tests: Urease test, Lactose test, Glucose Fermentation, TSI test, MR test, VP test, SIM test, Motility test, Indole test, Nitrate reduction test and Coagulase test to identify the organism to the species level.

Screening and extraction of EOs from selected plants Selection of plants and preparation of EOs under *in vitro* conditions: Plant based biocides as EOs (*viz.;* lemon grass, mint, black cardamom, clove, oregano) were taken to analyse their impact on food-borne microbes. EOs are also called aromatic volatile oils we get from plants and different parts of plants. Extraction of EOs can be done by steam distillation process which is very common as the extraction process for EOs. As we all EOs are a mixture of many chemical components. Each component has its valuable properties as an antibacterial, antiviral, anti-fungal, antioxidant and insecticidal which helps plants to protect from abiotic and biotic stress. They are produced from plants as glutinous concentrated extracts through steam distillation or by the combination of more than one method of extraction.

Bioefficacy of EOs against isolated microbes The effectiveness of the antibacterial agent was examined using the disc diffusion technique. Seven disks of equal size were placed on the Mueller Hinton agar plate. One disk on each plate served as the positive control, containing chloramphenicol (30 mg, 5 mm), while the other disks were impregnated with test samples that consisted of EOs. The anti-microbial activity of five EOs at different concentrations 20%, 40%, 60%, 80%, and 100% (v/v) was determined by appearance of the zone of inhibition against micro-organisms isolated from food samples. The essential oil's antimicrobial capacity was compared and characterized according to its antimicrobial effects [16].

Statistical analysis GraphPad Prism software was used to analyse the data collected, and multiple comparison procedures were run using One Way ANOVA, Holm-Sidak, and Tukey test. Mean and SD (Standard Deviation) are the outcomes. A *p*-value of less than 0.001 was considered statistically significant.

Results

Throughout the study (oranges, pomegranate, broccoli, banana, grapes, raw milk, potato, capsicum, cucumber, tomato, and sugarcane) were selected as raw food samples, and Tomato ketchup, green chutney, red chutney, orange juice, pani puri, pickle, curd, potato chips, chach, mix juice, papaya shake, pineapple juice, lemonade) selected as street food samples were collected from local vendors and microbiologically analysed presented in Table 1.

Isolation and identification of bacterial isolate In this study, we have identified 20 Gram-negative bacteria and 60 Gram-positive bacteria in the food samples that were collected. The qualitative and quantitative analysis (SCP-Standard Plate Count) was done to examine the diversity and number of microorganisms. A total of 20 gram-negative bacteria were isolated from various sources such as curd, potato, grapes, tomato ketchup, capsicum, chips, orange, banana, raw milk, orange juice, cucumber, broccoli, chach, papaya shake, and pineapple juice. On the other hand, 60 gram-positive bacteria were isolated from sources like orange, pomegranate, orange juice, broccoli, tomato ketchup, green chutney, red chutney, grapes, raw milk, panipuri, pickle, sugarcane, mixed juice, pineapple juice, chach, curd, and lemonade. The gram-negative bacteria showed maximum diversity with the isolation of *Enterobacter* sp., *Pseudomonas* sp., *Proteus* sp., *Acinetobacter* sp., *Citrobacter* sp., *Escherichia* sp., and *Moraxella* sp. while the gram-positive bacteria were identified as *Micrococcus* sp., *Bacillus* sp., *Staphylococcus* sp., *Lactobacillus* sp., and *Streptococcus* sp. These bacterial isolates were identified by Berger's Manual. The bacterial isolates which were identified with the percent incidence as *Staphylococcus* (57%), *Micrococcus* (30%), *Streptococcus* (7%), *Bacillus* (3%) *Lactobacillus* and *Neisseria* sp. (2%) as gram-positive bacteria and *Proteus* (25%), *Acinetobacter* (20%), *Pseudomonas* and *Enterobacter* (15%), *Escherichia* and *Citrobacter* (10%), *Moraxella* sp. (5%) as gram-negative bacterial isolates presented in Table 1 and Figure 1. We have selected 12 representative bacteria, 7 gram-negative (*Enterobacter, Pseudomonas, Proteus, Acinetobacter, Citrobacter, Escherichia, Moraxella* sp.) and 5 gram-positive bacteria (*Micrococcus, Bacillus, Staphylococcus, lactobacillus, Streptococcus* sp.) for the anti-microbial efficacy of EOs.

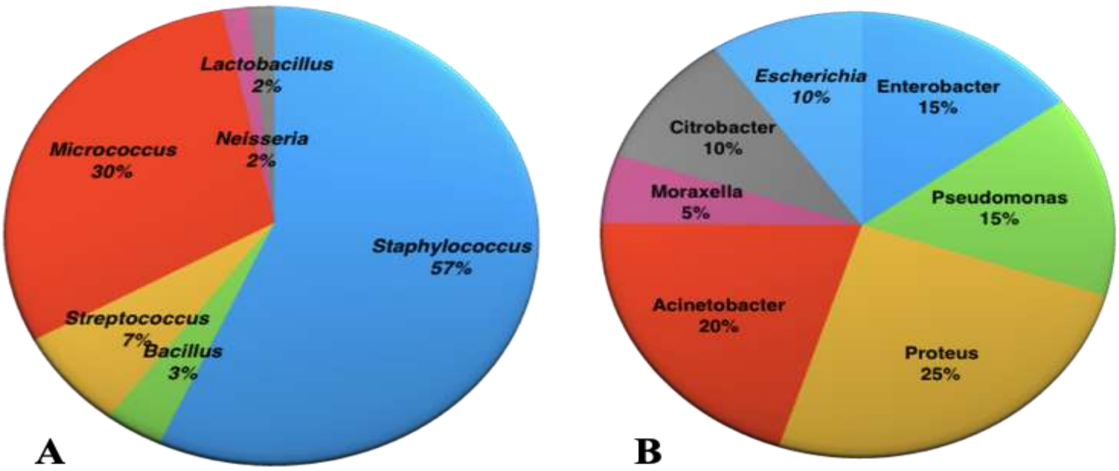
Table 1. Gram-negative and Gram-positive microbial isolates from Raw and Street food samples

Samples	Gram-negative isolates	Gram-positive isolates
Raw Food		
Orange	*Acinetobacter*	*Staphylococcus*
Pomegranate	*Acinetobacter*	*Bacillus*
Broccoli	*Proteus*	*Streptococcus*
Banana	*Proteus*	*Micrococcus, Staphylococcus*
Grapes	*Enterobacter*	*Micrococcus*
Raw milk	*Proteus*	*Staphylococcus*
Potato	*Enterobacter*	*Staphylococcus*
Capsicum	*Proteus*	
Cucumber	*Moraxilla*	*Micrococcus*
Tomato	—	*Neisseria*
Sugar Cane	—	*Staphylococcus*
Street Food		
Tomato ketchup	*Pseudomonas*	*Micrococcus*
Green chutney	—	*Micrococcus, Staphylococcus*
Red chuthey	—	*Staphylococcus*
Orange juice	*Acinetobacter*	*Staphylococcus*
Pani puri	—	*Staphylococcus*
Pickle	—	*Staphylococcus*
Curd	*Enterobacter*	*Micrococcus, Staphylococcus*
Chips	*Proteus*	*Staphylococcus*
Chach(lassi)	*Citrobacter*	*Micrococcus, Staphylococcus*
Mix can juice	—	*Micrococcus*
Papaya shake	*Escherichia*	*Streptococcus, Micrococcus*
Pineapple can juice	*Pseudomonas*	*Micrococcus, Staphylococcus*

Lemonade	—	*Streptococcus, Micrococcus, Staphylococcus*

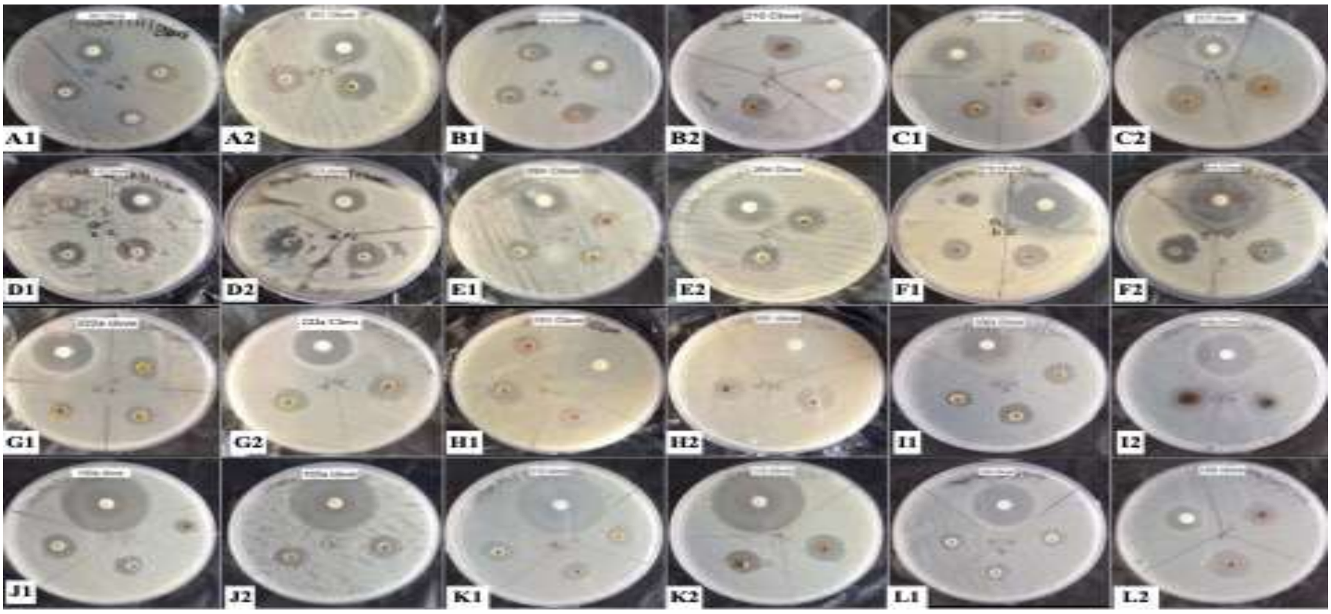
Note: ---- no growth

Figure 1. Representing Percent incidence of bacterial isolates from raw and street food samples, (A) Gram-positive, (B) Gram-negative



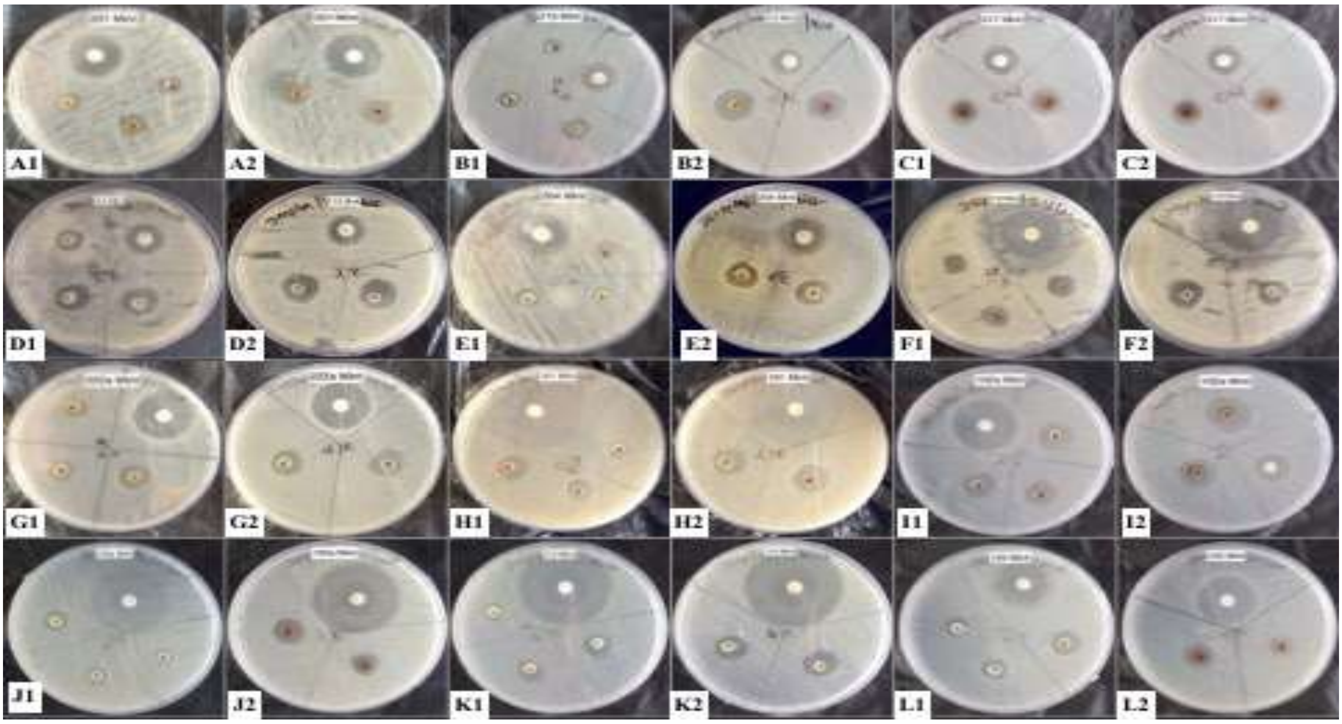
Bioefficacy of the EOs against selected bacterial isolates Five different EOs were tested for their antimicrobial properties at varying concentrations ranging from 20% to 100% (v/v). The oils tested were Clove, Oregano, Black Cardamom, Lemongrass, and Mint. Bioefficacy of EOs (EOs) was tested against isolates from food samples. Results showed that clove had the highest antimicrobial effect at all concentrations tested, followed by mint, black cardamom, oregano, and lemongrass which had the lowest antimicrobial effect, in comparison to Chloramphenicol (30 mg, 5 mm disc), which results as in positive control for all the bacterial isolates with the zone of inhibition (21-38 mm). Antimicrobial activity of these 5 EOs - lemongrass, clove, oregano, mint, and black cardamom was tested against 5 gram-positive bacteria *Micrococcus* sp., *Lactobacillus* sp., *Staphylococcus* sp., *Bacillus* sp. and *Streptococcus* sp. And 7 gram negative bacteria in which we have selected two isolates of *Proteus* sp., one is from raw food and another is from street food (*Enterobacter* sp., *Escherichia* sp., *Pseudomonas* sp., *Proteus* sp., *Acinetobacter* sp., and *Moraxella* sp.) isolated from food samples (Figure 2 and Figure 3). In order to determine the effectiveness of EOs in fighting both gram-positive and gram-negative bacteria, various concentrations of these oils were tested. The measurement of the zone of inhibition was recorded in millimeters for each essential oil tested. The study found that clove had the highest zone of inhibition (ranging from 14 to 18 mm), followed by mint (11 to 19 mm), black cardamom (12 to 18 mm), oregano (11 to 16 mm), and lemon grass (11 to 15 mm). These results were consistent across all microbial isolates and can be viewed in Table 2 and Figure 4.

Figure 2: Antibacterial activity of Clove EOs against microbial isolates



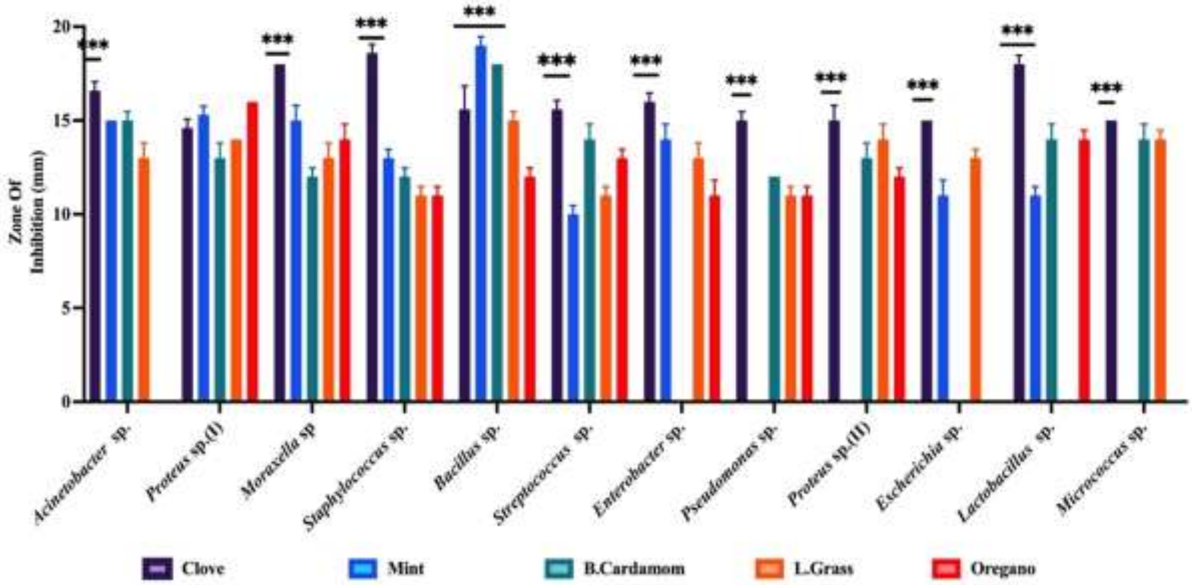
[A1,2 – *Acinetobacter*], [B1,2 – *Proteus* (I)], [C1,2 – *Moraxella*] [D1,2 - *Enterobacter*], [E1,2 - *Pseudomonas*], [F1,2 - *Proteus* (II)], [G1,2 - *Escherichia*], [H1,2 – *Staphylococcus*], [I1,2 – *Bacillus*], [J1,2 – *Streptococcus*], [K1,2 – *Lactobacillus*], and [L1,2 – *Micrococcus*], [a=20%, b=40%, c=60%, d=80%, e=100%, 0=Positive control (Chloramphenicol 30 mg)].

Figure 3: Antibacterial activity of Mint EOs against microbial isolates



[A1,2 – *Acinetobacter*], [B1,2 – *Proteus* (I)], [C1,2 – *Moraxella*] [D1,2 - *Enterobacter*], [E1,2 - *Pseudomonas*], [F1,2 - *Proteus* (II)], [G1,2 - *Escherichia*], [H1,2 – *Staphylococcus*], [I1,2 *Bacillus*], [J1,2 – *Streptococcus*], [K1,2 – *Lactobacillus*], and [L1,2 – *Micrococcus*], [a=20%, b=40%, c=60%, d=80%, e=100%, 0= Positive control (Chloramphenicol 30 mg)].

Figure 4: Mean and Standard Deviation of zone of bacterial growth inhibition (mm), resulting from the Disc Diffusion Method of five EOs against microbial isolates



Note:

- The bars in the figure represent the mean of the Zone of Inhibition and the error bars represent the standard deviation (SD). The method was conducted in set of triplets. Significance value at *p*<0.001.
- The following are the levels of significance and their corresponding *p*-values: **p*<0.05, ***p*<0.01, ****p*<0.001. Non-significant and denoted as 'ns'.

Table 2. Antimicrobial activity of EOs against microbial isolates associated with raw and street food

Organism	Control	L.Grass	Mint	B.Cardamom	Clove	Oregano
	Zone of Inhibition (mm)					
Raw Food						
Acinetobacter	21	13	15	15	16	—
Proteus (I)	21	14	15	13	14	16
Moraxilla	23	13	15	12	18	14
Staphylococcus	25	11	13	12	18	11
Bacillus	28	15	19	18	15	12
Streptococcus	35	11	10	14	15	13
Street Food						
Enterobacter	18	13	14	—	16	11
Pseudomonas	24	11	—	12	15	11
Proteus (II)	32	14	—	13	15	12
Escherichia	23	13	11	—	15	—
Lactobacillus	38	—	11	14	18	14
Micrococcus	25	14	—	14	15	—

Note:
- Chloramphenicol (30 mg, 5 mm disc) is used as a positive control,
- (—) shows least anti-bacterial effect, whereas >10 Sensitive, >12 Intermediate, >18 Resistant

Discussion

EOs are oily liquids extracted from various parts of plants. They are also known as volatile or ethereal oils and are highly aromatic. There is a rising appeal in employing essential oils (EOs) as natural food preservatives. However, food products need higher concentrations of EOs to exhibit their antimicrobial properties compared to in vitro studies. This is because the food matrix presents physical barriers [17]. The antimicrobial activity of EOs is intent on their composition, structure, and functional groups, which cause damage to bacterial cell membranes. Optimum hydrophobicity is also suggested to play a role in the toxicity of EOs [18]. During the present investigation, the antimicrobial activities of five EOs against 12 bacterial strains (7 gram-negative and 5 gram-positive) were evaluated at different concentrations. The essential oil of clove showed the maximum inhibition zone (~18mm) against Moraxella sp., and Staphylococcus sp. followed by mint possessing strong antimicrobial activity against Bacillus sp. with a zone diameter of 19mm. Cardamom also exhibited significant activity on Bacillus sp. with a zone size of 18mm. Among five EOs used the oregano showed the least activity on all the test isolates. The EOs of oregano, cardamom, and mint, showed very weak activity on Escherichia sp., and Micrococcus sp. respectively. According to a study, gram positive bacteria are more sensitive to oils compared to gram negative isolates. According to a number of scientific investigations, EOs exhibit antibacterial qualities against both gram-positive and gram-negative microorganisms. EOs such as clove, cinnamon, and rosemary have been reported to exhibit strong antimicrobial activity. The microbiological analysis of plant-based EOs has revealed a high microbial risk associated with fruits and vegetables. This can pose a serious health threat due to the presence of pathogenic microbial species such as Pseudomonas, Salmonella, Staphylococcus, Bacillus, and Escherichia sp. in green produce, even in the absence of contamination [19,20]. Similarly reports indicates that the raw food samples contained many pathogenic microbes, including gram-positive bacteria like Staphylococcus, Micrococcus, and Lactobacillus sp. and gram-negative bacteria such as Moraxella, Enterobacter, Pseudomonas, Neisseria, and Escherichia sp., which can cause foodborne illnesses [21,22]. A study demonstrated that volatile essential oil from lemongrass exhibits antimicrobial activity against S. aureus bacteria through the Disk Diffusion Method (DDM) or Turbidimetry Method (TM) [16]. Readymade EOs from the market (Oregano, Cinnamon, and thyme) show a successful antimicrobial effect on specific bacteria causing food contamination, due to the major components of Carvacrol, Cinnamaldehyde and thymol as their EOs [23]. EOs (clove, lemongrass, basil, thyme, oregano, marjoram and ginger) were examined on gram-positive (L. Monocytogene and S. aureus) and gram-negative (S. enteritidis, and E. coli) [24]. Crude olive oil extract with phenolic constituents has anti-microbial properties by controlling the rapid increase in the number of bacteria (E. coli, S. aureus, K. pneumonia) [25]. It is important to note that there is a growing need for innovative, natural, or eco-friendly ways to guarantee food safety. Using essential oils (EOs) as antibacterial additions is one suggested option. It's important to investigate this further to determine whether it could be a practical and secure substitute for conventional techniques.

Conclusion

Essential oils can serve as an effective alternate in the view of being used as preservatives. It will provide an effective tool and prevent adverse impact of chemical preservatives. Present study showcases the effectiveness of plant EOs in combatting foodborne microbes. The data indicated that clove exhibit statistically significant followed by mint and black cardamom against both gram positive and gram negative bacteria associated with row and street food. The study suggests recommendations for these essential oils to be used as preservatives followed by optimization as per the suggested guidelines to be included in the natural class of preservatives.

Conflict of Interest

The authors declare that they have no conflict of interests.

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