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ORIGINAL RESEARCH ARTICLE

Antibacterial Activity of Plant Essential Oils against Food Borne Bacteria

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ABSTRACT

The major aim of this study was to determine the antibacterial activity of plant essential oils against five food borne bacteria. The antibacterial activities of cinnamon, clove, oregano, rosemary and thyme oils were investigated against *Campylobacter*sp., *Listeria*sp., *Yersinia*sp., *Salmonella*sp.and *Pseudomonas* sp.by agar well diffusion method, minimum inhibitory concentration (MBC) and minimum bactericidal concentration (MBC) method. Most of the essential oils showed a relatively high antibacterial activity against all the food borne bacteria. Of the essential oils studied, clove, cinnamon and thyme are the more inhibitory activity against all five food borne bacteria. The ranges of MIC of the essential oils were 50 – 60, 60 – 80 and 80 – 100 µl ml^{-1}, respectively, for clove, cinnamon and thyme. This work shows that essential oil is more effective against food borne pathogens and spoilage bacteria and could be used as natural antibacterial agents in food preservation.

Key words: Antibacterial activity, Essential oils, Clove, Cinnamon, Thyme, Food borne bacteria.

1. INTRODUCTION

Illness caused due to the consumption of foods contaminated with pathogens such as *Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni*and *Salmonella* have a wide economic and public health impact worldwide [1]. These pathogens can adapt to survive and grow in a wide range of environmental conditions as well as in a large variety of raw and processed foods, including milk and dairy products such as cheese, yoghurt and butter. Food spoilage includes physical damage, chemical changes, such as oxidation, colour changes, or appearance of off-flavours and off-odours resulting from microbial growth and metabolism in the product [2]. The spoilage of milk and dairy products is caused by *Pseudomonas*species which are responsible for the off-odours, off-flavours, discolouration, gas production and slime production. The pseudomonads are also found in pasteurized milk and are generally from post-process contamination [3].

Current technologies for preservation and shelf life extension of food include chemical preservatives, heat processing, modified atmosphere packaging (MAP), vacuum packaging (VP) or refrigeration. Unfortunately, these steps do not eliminate undesirable pathogens such as *Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni*and *Salmonella* from these productsor delay microbial spoilage entirely. Alternative preservation techniques such as non-thermal technologies and naturally derived antimicrobial ingredients are under investigation for their application of food products.

Greater consumer awareness and concern regarding synthetic chemical additives have led researchers and food processors to look for natural food additives with a broad spectrum of antimicrobial activity [4]. In this context, plant essential oils are gaining interest for their potential as preservative ingredients or decontaminating treatments, as they have GRAS status and a wide acceptance from consumers [5]. The antimicrobial components are commonly found in the essential oil fractions and it is well established that many have a wide spectrum of antimicrobial activity, with potential for control of pathogens and spoilage bacteria within food systems [6, 7].

An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable. Leakage of ions and other cell

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contents can then occur. Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death [8, 9].Cinnamon (*Cinnamomumzeylanicum*), Clove (*Syzygiumaromaticum*), Oregano (*Origanumvulgare*), Rosemary (*Rosmarinusofficinalis*) and Thyme (*Thymus vulgaris*) are the most active essential oilsand display a good antimicrobial activity against Gram-positive and Gram-negative bacteria respectively.The present investigation was carried out, to study the antibacterial activity of plant essential oils against food borne bacteria isolated from dairy products.

2. MATERIALS AND METHODS

2.1. Essential oils

We used commercial essential oils ofCinnamon (*Cinnamomumzeylanicum*), Clove (*Syzygiumaromaticum*), Oregano (*Origanumvulgare*), Rosemary (*Rosmarinusofficinalis*) and Thyme (*Thymus vulgaris*). The essential oils were purchased from Cuddalore district, Tamilnadu, India.

2.2. Bacterial cultures

The bacterial strains *Campylobacter*sp., *Listeria*sp., *Yersinia*sp., *Salmonella*sp.and *Pseudomonas* sp. were isolated from dairy products such as raw milk, pasteurized milk, cheese, yoghurt and butter. Active cultures for experimental use were prepared by transferring a loopful of cells from stock cultures to flasks and inoculated in Brain Heart Infusion (BHI) broth medium at 37°C for 24 hours. Cultures of each bacterial strain were maintained on BHI agar medium at 4°C.

2.3. Antibacterial susceptibility test

Antibacterial activity of plant essential oils ofcinnamon, clove, oregano, rosemary and thyme were tested against five major food borne bacteria by Agar well diffusion method, Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC).

2.4. Agar well diffusion method

In order to determine the antibacterial spectrum, the antibacterial activity was performed by agar well diffusion method. The inoculum suspension of each bacterial strain was swabbed on the entire surface of Mueller Hinton Agar (MHA). On the surface of the medium, wells were made by using sterile cork borer (6 mm size). Each well was filled with 100 µl of essential oils. The well with sterile water served as control. The diameter of inhibition zones were measured in mm after incubation at 37°C for 24 hours. Three replications were maintained in each treatment.

2.5. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of the essential oils was tested by Tube dilution method. Mueller Hinton broth (MHB) was preparedand poured into sterile test tubes. Cell suspension (10^6 cells ml^{-1}) of the each bacterial strains*viz.*,*Campylobacter*sp., *Listeria*sp., *Yersinia*sp., *Salmonella*sp.and *Pseudomonas* sp.were prepared and added. The essential oils were added at different concentration *viz*., 400, 300, 200, 100, 90, 80, 70, 60 and 50 $\mu l ml^{-1}$. Then the tubes were incubated at 37°C for 24 hours. The lowest concentration of the essential oils at which the tested organism did not demonstrate visible growth was determined as MIC.

2.6. Determination of Minimum Bactericidal Concentration (MBC)

Plant essential oils that showed inhibitory activity in the preliminary broth assay were submitted to a subculture on the surface of the Mueller Hinton Agar (MHA) plates containing essential oils in the concentration of 200, 240, 320, 400, 800, 1,200 and 1,600$\mu l ml^{-1}$ in order to evaluate bacterial growth. The plates were then incubated for 24 hours at 37°C. MBC was defined as the lowest concentration of essential oils that kills 99.9% of the initial bacterial population where no visible growth of the bacteria was observed on the MHA plates.

2.7. Statistical Analysis

All experiments were done in triplicate, and mean values were calculated. The statistical analysis was carried out employing analysis of variance (ANOVA) (þ 0.05) using Completely Randomized Design. A statistical package (TNAUSTAT software) was used for the data analysis.

Table 1: Antibacterial activity of plant essential oils against food borne bacteria by Agar well diffusion method

Essential oils	Diameter of inhibition zone (mm)^a				
	***Campylobacter* sp.**	***Listeria*sp.**	***Yersinia*sp.**	***Salmonella* sp.**	***Pseudomonas* sp.**
Cinnamon	21.47	26.36	23.40	22.68	23.50
Clove	23.53	28.90	25.87	26.70	25.36
Oregano	18.00	21.17	20.56	25.37	20.23
Rosemary	17.26	20.00	18.63	19.17	18.67
Thyme	20.30	24.10	22.06	21.40	22.34
SEd	0.5157	0.9686	0.5561	0.5516	0.4753
CD (p=0.05)	1.1499	2.1600	1.2400	1.2300	1.0599

[a]Data are mean of triplicates; values are significantly different ($p < 0.05$) ; SEd- Standard error deviation; CD- Critical difference

Table2: Antibacterial effectof plant essential oils against food borne bacteria (Minimum Inhibitory Concentration method and Minimum Bactericidal Concentration method)

Organism tested	Essential oils (µl ml^{-1})									
	Cinnamon		Clove		Oregano		Rosemary		Thyme	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
*Campylobacter*sp.	80	320	60	240	400	1600	400	1600	100	400
Listeria sp.	60	240	50	200	200	800	300	1200	80	320
Yersinia sp.	60	240	50	200	300	1200	400	1600	80	320
Salmonella sp.	60	240	50	200	200	800	300	1200	80	320
Pseudomonas sp.	80	320	60	240	300	1200	400	1600	100	400

3. RESULTS AND DISCUSSION

According to the results of agar well diffusion method, MIC and MBC determination (**Table 1& 2**), essential oils of cinnamon, clove, oregano, rosemary and thyme had an inhibitory effect against food borne bacteria such as *Campylobacter*sp., *Listeria*sp., *Yersinia*sp., *Salmonella*sp.and *Pseudomonas* sp.Among all essential oils, clove oil exhibited the strongest antibacterial activity with significantly higher inhibition zone (23.53 – 28.90 mm) followed bycinnamon (21.47 – 26.36 mm) , thyme (20.30 – 24.10 mm), oregano (18.00 – 25.37 mm)and rosemary (17.26 – 20.00 mm) and the lowest MIC and MBC values.

Smith palmer *et al.* (2001)[10] reported the four essential oils of bay, clove, cinnamon and thyme can act as potent inhibitors of *Listeria monocytogenes* and *Salmonella enteritidis* in a food product.In our study *Listeria* sp.was found to be the most susceptible to clove, cinnamon, thyme, oregano and rosemary oils respectively. Vrindamenonand Garg (2001)[11] also reported the clove oil inhibited the*L.monocytogenes* in cheese at 1% concentration. The inhibitory activity of clove oil is due to the presence of some active components such as eugenol[12].Jagadeesh babu*et al.* (2011) [13]reported that clove, cinnamon and garlic had the antibacterial activity against various bacterial pathogens *Staphylococcus aureus*, *E.coli*, *Listeria monocytogenes, Bacillus cereus* and *Campylobacter jejuni*. Generally, the essential oils possessing the strongest antibacterial properties against food borne pathogens contain a high percentage of phenolic compounds such as carvacrol,cinnamaldehyde, eugenol and thymol.

Essential oils such as Clove (*Syzygiumaromaticum)* andCinnamon (*Cinnamomum zeylanicum)* showed antibacterial activity against food borne pathogens and have large spectrum activity due to their composition in phenolic compound. They constitute the more potential bio preservatives of food among these plants. Essential oils of cloveseem to be the most effective essential oil which is more active against the majority of bacteria and moulds which affect the quality of foods. Its activity is due to high concentration in eugenol, a phenolic component recognize to be active against more pathogens [14]. Shan *et al.* (2011)[15]investigated the antibacterial efficiency of five spice and herb extracts (cinnamon stick, oregano, clove, pomegranate peel, and grape seed) against *Listeria monocytogenes, Staphylococcus aureus,* and *Salmonella enterica* in cheese at room temperature (~23°C). The results showed that all five plant extracts were effective against three foodborne pathogens in cheese. The reduction of foodborne pathogen numbers indicated that the extracts of these plants (especially clove) have potential as natural food preservatives.

In this essential oils study, clove, cinnamon, thyme, oregano and rosemary are the more inhibitory activity against all five food borne bacteria *viz.,Campylobacter*sp., *Listeria*sp., *Yersinia*sp., *Salmonella*sp.and *Pseudomonas* sp. The ranges of MIC of the essential oils were 50-60, 60-80, 80-100, 200-400, 300-400 µl ml^{-1}, respectively, for clove, cinnamon, thyme, oregano and rosemary.Bayoub*et al*. (2010) [16] also studied the antibacterial effect of clove, cinnamon, thyme, rosemary, camomile extract against food borne pathogens.The MIC value between 0.25 mg/ml for clove extract and 6.75 mg/ml for camomile extract.In our study, the ranges of MBC of the essential oils were 200-240, 240-320, 320-400, 800-1600, 1200-1600 µl ml^{-1}, respectively, for clove, cinnamon, thyme, oregano and rosemary.

CONCLUSION

In conclusion, essential oils of clove, cinnamon, thyme, oregano and rosemary showed relatively high antibacterial activity against all the tested food borne bacteria. The present study suggests that the essential oil of clove, cinnamon and thyme is a potential source of natural antibacterial agents and to be used as food preservatives. After this screening experiment, phytochemical studies will be necessary to isolate the active constituents and evaluate the antibacterial activities against a wide range of bacterial population.

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