OCCURRENCE OF EMERGING FOOD BORNE PATHOGENS IN FAST FOODS AND ITS CONTROL BY ESSENTIAL OILS (EOs)

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Abstract

This investigation assessed the microbiological quality of various fast foods sold in Dhaka City, Bangladesh. Microbiological analysis was conducted on 55 samples, *viz*. beef burger, beef patties, egg chop, chicken patties, chicken shaworma, faluda, ice-cream, pasteurized milk, pudding and pastry. The isolates obtained from the samples were provisionally identified as *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli.* The highest level of *B. cereus* count (3.75 ± 0.31) was observed in faluda, while the highest levels of *S. aureus* and *E. coli* counts were observed (6.30 ±. 0.05) and (4.50 ± 0.24), respectively in chicken shaworma. Based on the attributes sampling planning for *S. aureus, B. cereus* and *E. coli,* all samples under study except ice-cream and pasteurized milk met no bacteriological quality standards, posing potential risk to consumers. Antibacterial activity of essential oils, such as cinnamaldehyde, euginol and carvacrol was carried out against the isolates of *B. cereus, S. aureus* and *E. coli* and from the experimental findings it was apparent that the essential oils showed antibacterial activity against the pathogens tested. The Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) values of the essential oils against the test bacteria ranged from 2.5 to 10% and 5 to 15%, respectively. Essential oils can be used as alternatives of antibiotics and / or chemical preservatives in the fast foods.

Key words: Pathogens, fast foods, essential oils, cinnamaldehyde, carvacrol, euginol

INTRODUCTION

Over the past 10-15 years, the main trends in food consumption worldwide have been increased as pre-prepared food in the home and outside the home particularly in fast food restaurants (Gould and Bowie 1952). Food borne pathogens are the leading cause of illness and death in less developed countries killing approximately 1.8 million people per annum. In developed countries, food borne pathogens are responsible for millions of cases of infectious gastrointestinal diseases every year, costing billions of dollars for medical care and lost productivity (Fratamico and Bayles 2005). The presence of microorganisms in food is a natural and unavoidable occurrence. Culinary cooking generally destroys most harmful bacteria, but undercooked foods, processed ready to eat foods and minimally processed foods may contain harmful bacteria that are serious health threats. In spite of modern improvement in hygiene and food production techniques, food safety is an increasingly important public health issue (WHO 2002). It has been estimated that as many as 30% of people in industrialized countries suffer from food borne disease every year and in 2000 at least two million people died of diarrheal disease worldwide (WHO 2002).

The interest in essential oils and their application in food preservation have been amplified in recent years by an increasingly negative consumer perception of synthetic preservatives. Now-a-ways, consumers have growing concern about the food safety in relation to hormones, food additives, food preservatives etc (Brewer *et al*. 1994). The need for safe antimicrobial preservatives increased as alternatives of chemical preservatives in foods. For the replacement of synthetic antimicrobial additives

active components from herbs and spices are to be effective alternatives today (Brewer *et al*. 1994). For this reason, many studies on antimicrobial substance of spices as a natural food preservative against food borne pathogens have been reported (Smith *et al.*1998).

Spices and herbs have been used traditionally for several centuries by many cultures in preserving foods and as food additives for aroma (Shelef 1983, Zaika 1988). Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs and their components (Shelef 1983, Zaika 1988). Previous investigation confirmed that garlic, onion, cinnamon, cloves, thyme, sage, and other spices inhibit the growth of both Gram-positive and Gram-negative food borne pathogens or spoilage bacteria, yeast, and molds. It has long been recognized that some EOs have antimicrobial properties (Boyle 1955) and these have been reviewed in the past (Shelef 1983, Nychas and Skandamis 2003) as antimicrobial properties of spices (Boyle 1955).

Main antimicrobial component in cinnamon has been reported to be cinnamaldehyde which has been given special attention to find their antibacterial activity against food borne pathogens. Cinnamaldehyde acts on both Gram-positive and Gram-negative bacteria, including the organisms that are food safety concern. Cinnamaldehyde has been reported to inhibit the growth of *C. botulinium* (Bowles and Miller 1993), *S. aureus*, *E. coli* 0157:H7 and *Salmonella* serovar *typhimurium* (Helander *et al*. 1998).

S. aureus, *B. cereus* and *E. coli* are frequently reported food contaminant and is commonly recovered from ham, egg, tuna, chicken, potato, and macaroni, bakery products, such as cream-filled pastries, cream pies, and chocolate products, sandwiches, undercooked ground beef. Other sources include milk and dairy products, as well as meat, poultry, eggs, and related products. Convenience food offers a suitable growth environment for toxin-producing bacteria such as *Staphylococcus aureus*, which is able to grow and express virulence in a wide variety of foods, such as milk products, mixed foods, meat and meat products, egg and egg products, cakes and ice cream (Adams and Moss 2008).

The objective of this study was to assess 1) Isolation of food borne pathogens from common fast food samples in Dhaka, 2) Identify the pathogens by conventional microbiological method. 3) Screening of the essential oil for antibacterial activity against food borne pathogens. 4) Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

MATERIAL AND METHODS

Sample collection

Food samples were collected from different areas of Dhaka City (Table 1). For the purpose of sampling some clean dry, sterile zip lock bags were taken to the sampling site. About 100g of food sample was collected for each test and the samples were kept in the bags. The samples were transferred to the laboratory as soon as possible.

Medium for isolation of E. coli, B. cereus and *S. aureus*

For the isolation of *E. coli, B. cereus* and *S. aureus* from the food samples, three types of selective media were used, such as Sorbitol MacConkey agar (SMA), *Bacillus cereus* selective agar base medium supplemented with egg yolk and Polymyxin B, and Mannitol salt agar (MSA). Nutrient agar medium was used for sub-culturing the isolates.

Table 1. Categories of food samples and sampling sites at Dhaka City.

Name of the sample	Site of collection
Beef Burger	New market,Gulshan
Beef patties	New market
Egg chop	Chandi Chalk
Chicken sandwich	New market
Chicken patties	New market
Chicken shaworma	Mirpur
Faluda	Gulshan
Ice-cream	New market
Pudding	Mirpur
Pastry	Mirpur, New market

Preparation of food suspension

Ten g of each food sample were taken into a 250 ml conical flask containing 100 ml of sterilized physiological saline and homogenized with a homogenizer (NISSEI, Japan) for one minute. The resulting suspension was diluted in sterile normal saline (physiological saline) following the tenfold serial dilution technique suggested by Cappuccino and Sherman (1999).

Inoculation and incubation

A sample suspension of 0.1 ml from each dilution (original to 10^{-5}) was transferred onto selective agar plates with sterile micropipette tips and spread uniformly over the agar surface by sterile spreaders. In this way three sets of plates for each dilution of each sample were made. Three sets of plates were incubated at 37°C for 24h.

Isolation of colonies

From the selective medium, the discrete selected colonies were obtained and then sub-cultured onto nutrient agar plates to obtain pure culture. From the collected food samples, 14 *E. coli*, nine *B. cereus* and 17 *S. aureus* isolates were subjected to cultural, morphological, microscopic and biochemical studies for presumptive identification process.

Taxonomic study

For this purpose an array of microscopic and biochemical tests and fermentation tests were performed that served for the identification of the isolates (John 1994).

Reference strains and maintenance of the culture

A total of three strains or species of frequently reported food borne pathogens such as *E. coli* ATCC 25923, *B. cereus* ATCC 11778, and *S. aureus* ATCC 25922 were used in this study. The long time stock cultures of the test organism in 20% glycerol in cryogenic vials were kept at -84° C. Working cultures were kept at 4°C on Tryptone soya agar slants (Nissui, Japan) and were periodically transferred to fresh slants.

Essential oil

The essential oils that were used in this study were cinnamaldehyde, carvacrol and euginol. Cinnamaldehyde was purchased from Nacalai Tesque Co (Kyoto, Japan) and carvacrol and euginol from Wako Pure Chemical Industries Limited (Osaka, Japan).

Antimicrobial activity testing

The antimicrobial activity of cinnamaldehyde, carvacrol and euginol was determined according to the method of Bauer *et al*. (1966). Eight mm in diameter discs (ADVANTEC Toyo Roshi Kaisha, Ltd. Japan) were impregnated with 50 µl of 3% of each essential oil before being placed on the inoculated agar plates. The inocula of the test organisms were prepared by transferring a loopful of culture into 9 ml of sterilized Mueller Hinton Broth (MHB) (Difco) and incubated at 37°C for 5 to 6h, if necessary 12 to 18h. The MHB culture was compared with McFarland 0.5 (Jorgensen *et al.* 1999) turbidity standards (10^7 CFU/ml) and streaked evenly in three plates with the cotton swab at a 60° angle on the surface of the Mueller Hinton Agar (MHA) plate. Excess suspension was removed from the swab by rotating it against the side of the tube before the plate was seeded. After the inocula dried, the impregnated discs were placed on the agar using a forceps dipped in ethanol and flamed, and were gently pressed down to ensure contact. Plates were kept at 4°C for 30 to 60 min for better absorption, during this time microorganisms not grow, but absorption of the extracts would take place. Negative control was prepared using the same solvent without the essential oil. A reference antibiotic, imipenem, was used as a positive control. The inoculated plates containing the impregnated discs were incubated in an upright position at 37°C overnight for 24 to 48h. The results were expressed as the zone of inhibition around the discs (8 mm).

Determination of minimum inhibitory concentration

The essential oils, which exhibited the best antimicrobial activity in the paper disk diffusion assay were selected for determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using broth dilution method (a modified method of Hasika *et al*. 2014). One colony of each bacterial isolate was sampled with a loop, then inoculated in 25 ml MHB and incubated for 18–24h at 37°C in order to get a bacterial suspension of 10^9 cfu/mL. 0.9 ml of the MHB was taken in each of the sterile and dry glass vials appropriately labeled with concentration of essential oil. 1.0 ml of the respective essential oil concentration was dispensed into the respective vials and 100 µl of each test organisms were added to the vials with their names labeled to make sure that each of the organisms faced a different concentration of the essential oil. So, the final reaction volume became two ml. A negative control was made with essential oil and MHB but no test organisms. All the prepared vials were then incubated at 37°C for 24h. Because when oils were diluted in buffer and media became turbid for this growth was not visible in the MHB. For determination of MIC, 10 µl inocula from each test vials were transferred onto MHA plate by a micropipette. The plates were then incubated at 37°C for 24h. The MIC was considered as the lowest concentration that prevented the visible growth.

Minimum bactericidal concentration (MBC)

The tubes of MIC that showed no growth of the microbes were sub-cultured by streaking using sterile loop on nutrient agar plates. The plates were incubated at 37°C for 24h. The MBC represents the lowest concentration of the extract that did not show any colony on plates (Spencer and Spencer 2004).

Statistical analysis

The inhibition zone was calculated as means±SD (n=3 or 4). The significance among different data was evaluated by analysis of variance (ANOVA) using Microsoft excel program. Significant differences in the data were established by least significant difference at the 5% level of significance. The inactivation *of E. coli*, *B. cereus* and *S. aureus* experiments were done two times with triplicate samples being analyzed at each sampling time. Levels were expressed as the log CFU per gram recovered by direct plate counts.

RESULTS AND DISCUSSION

Suspected E. coli

Based on the characteristic colony of selective medium, the suspected *E. coli* colony prevalence is shown in Table 2.

Table 2. Prevalence of suspected *E. coli* (considering the colony morphology) in food samples.

Name of the samples	Sample size	Range of counts/ml or g	Average counts/ml or g	Mean ± SD
Beef burger	5	1.7×10^1 - 4.0×10^1	2.82×10^1	1.45 ± 0.14
Beef patties	5	2.9×10^3 - 5.3×10^3	4.10×10^3	3.61 ± 0.11
Chicken sandwich	5	2.6×10^3 - 1.35×10^5	1.92×10^4	4.28 ±0.34
Chicken shaworma	5	1.13×10^4 - 5.5×10^4	3.23×10^4	4.50 ± 0.24
Ice cream	5	0	0	0
Pasteurized milk	5	0	0	0

Suspected S. aureus

Based on the characteristic colony of selective medium, the suspected *S. aureus* colony prevalence is shown in Table 3.

Table 3. Prevalence of suspected *Staphylococcus aureus* (considering the colony morphology) in food samples.

Name of the samples	Categories of samples	Sample size	Range of counts/ml or g	Average counts/ml or g	Mean ± SD
Milk based products	Raw milk	5	5.0×10^4 - 9.2×10^4	6.6×10^4	4.82 ± 0.1
	Ice cream	5	0	0	0
	Pasteurized milk	5	0	0	0
Egg-milk based product	Pudding	5	1.2×10^3 - 2.8×10^3	2.2×10^3	3.34 ± 0.15
Beef products	Beef burger	5	6.0×10^4 - 2.0×10^6	3.49×10^5	5.54 ± 0.58
Chicken based products	Chicken sandwich	5	8.0×10^3 - 1.3×10^5	1.41×10^4	4.14 ± 0.29
	Chicken shaworma	5	1.0×10^4 - 8.0×10^6	2.12×10^5	6.32 ± 0.51
	Chicken patties	5	7.0×10^4 - 1.0×10^5	6.5×10^4	4.81 ± 0.13

Suspected B. cereus

Based on the characteristic colony of selective medium, the suspected *B. cereus* colony prevalence is shown in Table 4.

Table 4. Prevalence of suspected *Bacillus cereus* in milk based food samples.

Name of the samples	Sample size	Range of counts/ml or g	Average counts/ml or g	Mean ± SD
Egg chop	5	2.0×10^2 - 5.0×10^2	2.5×10^2	2.47 ± 0.27
Faluda	5	1.0×10^3 - 5.0×10^3	3.19×10^3	2.44 ± 0.31
Pastry	5	0.1×10^2 - 0.3×10^2	1.1×10^2	2.04 ± 0.08
Ice cream	5	0	0	0
Pasteurized milk	5	0	0	0

International Commission on Microbiological Specifications for Foods (ICMSF 1986) supports the attribute sampling plans, which are to be applied for the determination of quality assurance of the test samples:

Attribute sampling plans for *S. aureus* allow two class plans which include the following formula for safe food:

$N = 5$, $c = 0$ and $m = 1 \text{ X } 10^3$/g or ml

Where n = number of sample units from a lot**,** c = maximum number of sample units that may exceed the value of m, and m = maximum number of relevant bacteria/g or ml. Results are shown in Table 5.

Table 5. Analysis of quality assurance of the test samples.

Category of food samples	Numbers of Sample (*n*)	Presence of *S. aureus* in the samples (*c*)	Maximum count of *S. aureus* (*m*) cfu/g or ml	Comment
Raw milk	5	5	$9.2 \text{ x } 10^4$	Unaccepted
Ice cream	5	0	0	Accepted
Pasteurized milk	5	0	0	Accepted
Pudding	5	5	$2.8 \text{ X } 10^3$	Unaccepted
Beef burger	5	5	$2.0 \text{ X } 10^6$	Unaccepted
Chicken sandwich	5	5	$1.3 \text{ X } 10^5$	Unaccepted
Chicken shaworma	5	5	$1.0 \text{ X } 10^6$	Unaccepted
Chicken patties	5	5	$1.0 \text{ X } 10^5$	Unaccepted

S. aureus on all samples except ice cream and pasteurized milk samples showed higher counts than the acceptable levels ($n = 5$, $c = 0$, or $m = 10^3$). All samples except ice cream and pasteurized milk samples did not meet bacteriological quality standards.

Attribute sampling plans for *B. cereus* allow two class plans which include the following formula for safe food:

$N = 5$, $c = 0$ and $m = 10^3$/g or ml

Table 6. Analysis of quality assurance of the test samples.

Category of food samples	Numbers of Sample (*n*)	Presence of *B. cereus* in the samples (*c*)	Maximum count of *B. cereus* (*m*) cfu/g or ml	Comment
Egg chop	5	5	$5.0 \text{ X } 10^2$	Accepted
Faluda	5	5	$5.0 \text{ X } 10^3$	Unaccepted
Pastry	5	5	$0.3 \text{ X } 10^2$	Accepted
Ice cream	5	0	0	Accepted
Pasteurized milk	5	0	0	Accepted

Presence of *B. cereus* on the samples tested contains 10^3/g or less ($c = 0$), then the egg, pastry, ice cream and pasteurized milk samples were accepted, but faluda samples showed *B. cereus* counts more than 10^3/g, did not meet bacteriological quality standards.

Attribute sampling plans for *E. coli* allow three class plans which include the following formula for safe food:

$n = 5$; $c = 2$; $m = 10^1$; $M = 10^2$

Hence, the three class plan is where the food can be divided into three classes according to the concentration of microorganisms detected:

Acceptable' if counts are below *m*.

Marginally acceptable' if counts are above *m*, but less than *M*.

Unacceptable' if counts are greater than *M*.

M = The count above which the lot is unacceptable.

From the Table 7, it is apparent that two samples out of five (beef burger) contained between 10^1 and 10^2 *E. coli* and be acceptable. However, other food samples contained *E. coli* greater than *M* (10^2) and did not meet bacteriological quality standards.

Table 7. Analysis of quality assurance of the test samples.

Category of food samples	Numbers of Sample (*n*)	Presence of *E. coli* in the samples (*c*)	Minimum count of *E. coli*(*m*) cfu/g or ml	Maximum count of *E. coli*(*M*) cfu/g or ml	Comment
Beef burger	5	5	1.7×10^1	4.0×10^1	Accepted
Beef patties	5	5	2.9×10^3	5.3×10^3	Unaccepted
Chicken sandwich	5	5	2.6×10^3	1.35×10^5	Unaccepted
Chicken shaworma	5	5	1.13×10^4	5.5×10^4	Unaccepted
Ice cream	5	0	0	0	Unaccepted
Pasteurized milk	5	0	0	0	Unaccepted

The stringency of the sampling plan can be decided using the ICMSF (1986) concept based on the hazard potential of the food and the conditions which a food is expected to be subject to before consumption.

Screening of the essential oil

Results for screening of antibacterial activity of cinnamaldehyde, euginol and carvacrol are summarized in Table 8.

Table 8. Antibacterial activity of cinnamaldehyde, euginol and carvacrol against food borne pathogens.

Isolates	Zone of inhibition (in mm)			
	CNN 3%	EU 3%	CAR 3%	Standards(IPM) (10 µg)
Ec 0	28.8 ± 1.0	17.0 ± 1.3	23.0 ± 1.3	17.0 ± 1.0
Ec 01	28.5 ± 0.70	18.0 ± 2.0	21.0 ± 1.0	15.5 ± 0.5
Ec 07	29.0 ± 1.41	17.5 ± 1.5	24.0 ± 2.0	15.5 ± 1.5
Ec 17	29.0 ± 1.41	15.5 ± 0.5	24.0 ± 1.0	19.0 ± 1.0
Bc 0	32.5 ± 0.8	20.0 ± 0.5	24.5 ± 1.0	23.5 ± 1.5
Bc 01	30.5 ± 0.5	23.5 ± 0.5	27.0 ± 1.0	26.0 ± 2.0
Bc 02	31.0 ± 1.0	21.0 ± 1.0	20.0 ± 2.0	22.5 ± 2.5
Bc 06	33.0 ± 1.0	19.5 ± 0.5	23.0 ± 1.5	22.5 ± 2.5
Bc 10	34.5 ± 0.5	20.5 ± 0.5	18.5 ± 1.5	22.5 ± 1.5
Bc 12	33.0 ± 1.0	19.5 ± 0.5	26.5 ± 1.5	21.5 ± 2.5
Sa 0	37.0 ± 1.5	25.0 ± 1.0	25.5 ± 1.0	27.0 ± 1.5
Sa 05	38.0 ± 2.0	19.0 ± 1.0	25.0 ± 1.0	17.0 ± 1.0
Sa 08	38.0 ± 2.0	23.5 ± 0.5	18.0 ± 2.0	27.5 ± 2.5
Sa 09	34.0 ± 2.0	24.5 ± 1.5	22.0 ± 2.0	26.5 ± 1.5
Sa 12	37.0 ± 1.0	22.5 ± 0.5	29.0 ± 1.0	32.0 ± 2.0
Sa 22	39.0 ± 1.0	27.0 ± 1.0	25.5 ± 1.5	25.5 ± 1.5

Reference strains: Ec 0, *E. coli* ATCC 25923; Bc 0, *B. cereus* ATCC 11778; Sa 0, *S. aureus* ATCC 25922; Mean diameter of zone of inhibition in mm including the diameter of the disc (8 mm) CNN stands for cinnamaldehyde, EU stands for euginol and CAR stands for Carvacrol represents Mean ± S.D (n=2) IPM Imipenem

Cinnmaldehyde showed good inhibitory activity against the three food borne pathogens with zones of inhibition ranging from 28 to 39 mm. It showed maximum inhibition for *S. aureus* (39 mm) and minimum inhibition for *E. coli* (28.5 mm) with the zones of inhibition larger than those observed against

the antibiotic Imipenem (10 µg). Euginol showed maximum inhibition for *S. aureus* (27 mm) and minimum inhibition for *E. coli* (15.5 mm) and carvacrol showed maximum inhibition for *S. aureus* (29 mm) and minimum inhibition for *E. coli* (18 mm). the antibacterial activity of the EOs was carried out against the reference strains (viz. *E. coli* ATCC 25923, *B. cereus* ATCC 11778, and *S. aureus* ATCC 25922) and from the Table 8, it is found that there was no significant antibacterial potentiality difference amongst the reference strains and the isolates used in the study.

MIC and MBC of cinnamaldehyde, carvacrol and euginol

MIC and MBC of cinnamaldehyde, carvacrol and euginol (ranged from 10 to 0.03125%) were determined by broth dilution method at 37°C against the test organisms with reference strains. Only MIC and MBC values are shown in Table 9.

Table 9. MIC and MBC of cinnamaldehyde (CNN), carvacrol (CAR) and euginol (EU) against food borne pathogens.

Isolates	**Essential oils**					
	CNN		CRA		EU	
	MIC	MBC	MIC	MBC	MIC	MBC
EC 0	2.5	5	2.5	5	2.5	5
Ec 01	2.5	5	2.5	5	2.5	5
Ec 07	2.5	5	2.5	5	2.5	5
Ec 17	2.5	5	2.5	5	2.5	5
Bc 0	2.5	5	5	10	5	10
Bc 01	2.5	5	5	10	10	15
Bc 02	2.5	5	2.5	5	5	10
Bc 06	2.5	5	5	10	5	10
Bc 10	2.5	5	5	10	2.5	5
Bc 12	2.5	5	2.5	5	2.5	5
Sa 0	5	10	5	10	5	10
Sa 05	5	10	5	10	5	10
Sa 08	10	15	5	10	10	15
Sa 09	2.5	5	2.5	5	2.5	5
Sa 12	10	15	10	15	5	10
Sa 22	10	15	5	10	5	10

Reference strains: Ec 0, *E. coli* ATCC 25923; Bc 0, *B. cereus* ATCC 11778; Sa 0, *S. aureus* ATCC 25922

All the isolates of *E. coli* were Gram negative, non-spore forming short rods; *S. aureus* were Gram positive, non-spore forming cocci shaped, and *B. cereus* cells were Gram positive, spore forming long rod shaped bacteria.

E. coli was positive for MR, indole and catalase tests, and negative for VP, citrate, urease, H_2S, gelatin, and oxidase tests; *S. aureus* was positive for MR, VP, and catalase tests but negative for oxidase, indole, starch and urease tests, and able to ferment carbohydrates such as lactose, sucrose with acid production only and negative response to H_2S production; *B. cereus* was positive for VP, nitrate, citrate, catalase and gelatin hydrolysis tests and negative for MR, and indole tests. All isolates of *B. cereus* showed positive response to glucose and fructose and negative response to arabinose, rhamnose, galactose, and xylose, but variable response to mannose.

In this study the antibacterial activity of cinnamaldehyde, carvacrol and euginol was determined against *E. coli*, *S. aureus* and *B. cereus* by the disc diffusion method. The results presented in Table 8 showed that the oils under investigation exhibited marked antibacterial activity against all the test organisms. The findings of cinnamaldehyde correlated with the findings of Hoque *et al*. (2008) who applied the essential oils of cloves and cinnamon against food borne pathogens and spoilage bacteria.

The essential oils under this study consisted of phenolic components, which rendered them effective against the tested microorganisms. This was confirmed by Farag *et al*. (1989).

The diameters of the zones of inhibition of cinnamaldehyde obtained by disc diffusion method were compared to those obtained against commonly used antibiotic Imipenem (10 µg/disc) which was used as standard (Table 8). Cinnamaldehyde showed maximum inhibition for *S. aureus* (39 mm) and minimum inhibition for *E. coli* (28.5) with zones of inhibition larger than those observed against the Imipenem (10 µg/disc). In comparison to antibacterial activity of the three essential oils studied, cinnamaldehyde was found more potential. These findings have correlated with the reports of Hoque *et al*. (2008).

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