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In-vitro assessment of antimicrobial and antioxidant potential of essential oils from Lemongrass (*Cymbopogon citratus)*, Cinnamon (*Cinnamomum verum*) and Clove (*Syzygium aromaticum*)

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ABSTRACT

The present study was envisaged to investigate *in-vitro* antimicrobial and antioxidant efficacy of lemongrass, cinnamon and clove essential oils for their potential application in meat products. Antimicrobial potential was measured by using zone inhibition assay and Minimum Inhibitory Concentration (MIC) against foodborne pathogens including Gram positive and Gram negative whereas, antioxidant assay was done using DPPH and ABTS radical scavenging activity. MIC values of lemongrass oil ranged from 500-3000 ppm and was found more effective against Gram positive than Gram negative organisms whereas cinnamon as well as clove oil were effective against both the classes of organisms. ABTS and DPPH radical scavenging activity of all the three different oils were measured at five different concentrations and as per MIC values, the DPPH values were ranging from 38.05- 48.45% whereas ABTS values ranged from 25.17-45.66% for three oils under investigation. It is concluded that these essential oils possess potent antimicrobial and antioxidant activity and can be used as a natural alternative for preservation in meat industry.

Keywords: Lemongrass, cinnamon, clove, antimicrobial, DPPH, ABTS

Being natural source of various components with multitude of biological actions, plant derived active principles are gaining importance in food industry. In recent times, the popularity for use of essential oils and medicinal extracts has increased worldwide (Velázquez-Nuñez *et al.,* 2013). This could be due to quest of consumers for a preservative in their foods with minimum residual effect (Mehta *et al.,* 2015). An increased incidence of undesirable aspects like carcinogenicity, teratogenicity and slow degradation is largely associated with the use of chemicals for control of food spoilage like synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Olmedo *et al.,* 2014). The awareness regarding possible health risks due to synthetic chemicals used in foods have reinforced the search for natural sources of antimicrobials and antioxidants. Their replacement with relatively much lesser hazardous compounds has steered the research in food industry to a new level. Thus, the role of phytochemicals and phyto extracts for inclusion in food for enhancement of preservative functionality is gaining momentum. Even the essential oils or compounds obtained from plants have gained acceptance by industries, owing to their little or no harm on human health. So, these can be used as a potential substitute to synthetic antimicrobials and antioxidants.

Essential oils are not strictly oils and possess pleasant odour and distinctive taste (Burt, 2004). Presence of bioactive volatile compounds of different chemical identities is responsible for the antimicrobial or other biological activities of these oils (Mahmoud and Croteau, 2002). It may be terpene compounds (mono-, sesqui- and diterpenes), alcohols, acids, esters, epoxides, aldehydes, ketones, amines and sulfides (Bakkali *et al.,* 2008) which are reported to be effective antimicrobials against

a number of foodborne pathogens including *E. coli*, *Salmonella Typhimurium, S. aureus, L. monocytogenes, Campylobacter* and others (Callaway *et al.,* 2011). The degree of inhibition is dependent on type and concentration of oil used. Lemongrass essential oils possess potent activity due to presence of citral (geranial and neral) which constitutes nearly 75-80% of total compounds in the oil (Kakrala and Ganjewala, 2009). Due to strong action against variety of microorganisms, it is visualized as a potent alternative for synthetic antimicrobials. Though it has medicinal properties and is safe; its application in meat industry is limited and not much work has been carried out. The active constituent in Cinnamon essential oil is cinnamaldehyde that is reported to possess antibiotic quality (Wong *et al.,* 2014). Eugenol (4 allyl-2-methoxy phenol; $C_{10}H_{12}O_2$) an organic phenol compound is the major constituent of clove essential oil (Shahavi *et al.,* 2015) and reported to be the main antimicrobial constituent (Hoque *et al.,* 2008). Biological activities of these essential oils as antifungal, antioxidant, and antimicrobial have been studied but their application in food particularly meat is a new area to investigate (Espina *et al.,* 2011; Jing *et al.,* 2014; Liu *et al.,* 2012; Singh *et al.,* 2010). With this background, the aim of this study was to investigate and evaluate the in-vitro antimicrobial and antioxidant potential of lemongrass, cinnamon and clove essential oils for their possible application in meat products.

MATERIALS AND METHODS

Source of lemongrass, cinnamon and clove essential oils

Lemongrass, cinnamon and clove oil were procured from Kanta Enterprises Pvt. Ltd., Noida, UP, India. The certificate of analysis provided with the product is mentioned in Table 1. All the reagents and chemical used in the study were of analytical grade.

Table 1: Physico-chemical parameters of lemongrass, cinnamon and clove oil

Physico chemical properties	Lemongrass Oil	Cinnamon Oil	Clove oil
1. Specific Gravity	0.894-0.924	1.010-1.030	1.038-1.060
2. Refractive Index	1.483-1489	1.508-1.591	1.527-1.535
3. Main constituent by GC	Citral 70% +	Cinnamalde-hyde 85%	Eugenol 82%+

Bacterial strains and growth conditions

Nine pure freeze dried cultures were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India viz. *Salmonella enterica* (MTCC 3231), *Escherichia coli* (MTCC 2991), *Staphylococcus aureus* (MTCC 7443), *Shigella flexneri*, *Yersinia enterolitica* (MTCC 3238), *Klebsiella pneumoniae* (MTCC 9544), *Vibrio parahaemolyticus* (MTCC 451), *Listeria monocytogenes* (MTCC 657) and *Bacillus cereus* (MTCC 6728). These cultures were revived and stock cultures were prepared and are being maintained in the department by regular passaging.

Antimicrobial activity estimation

Antimicrobial potential of Lemongrass, Cinnamon and Clove essential oil was tested on nine entero-pathogenic bacterial cultures (*Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Salmonella typhi, Staphylococcus aureus, Shigella flexneri, Vibrio parahaemolyticus and Yersinia enterocolitica*), which were cultured into fresh broth media and brought into log phase of growth by incubating at 37° C for 24 hours before being used. The antibacterial activity was assessed using well diffusion method. Petri plates were poured with sterilized nutrient agar and left undisturbed for 36 hours and wells of 10 mm diameter were bored in the agar plates using sterile cork borer. The plates were inoculated with bacterial cultures by spread plating 100 µl of each bacterial culture. 100 µl of Lemon Grass, cinnamon and clove oil was poured into each of the wells and the plates were incubated at 37° C for 24 hours; observed for appearance of zones of bacterial growth inhibition around the oil containing wells. Diameters (mm) of these zones were measured.

Minimum inhibitory concentration (MIC)

Uniform concentrations of log phase bacterial cultures were prepared by adjusting their absorbance at 600 nm. Concentrations of Lemongrass, Cinnamon and clove essential oil were adjusted with the help of DMSO. In the Microtiter plates, 100 µl of each culture were added to 30 µl of oil dilution and 170 µl of nutrient broth. The plates were kept for incubation at 37° C for 24 hours. Absorbance of samples was measured at 600 nm to observe growth

inhibition. The growth inhibition was also confirmed by streaking the samples on nutrient agar plates and observing for bacterial growth after 24 h incubation at 37° C.

Antioxidant activity assay

The antioxidant activity of the oils was measured using the 2, 2′-azinobis-3-ethylbenzthiazoline-6-sulphonate (ABTS) radical and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. DPPH radical scavenging activity of Lemon Grass, Cinnamon, and clove oil was assessed using a methanolic solution of the "stable" free radical, DPPH•. The method of Blois (1958) was used in studying the effect of various oil concentrations on DPPH• radicals with some modifications. A solution of DPPH• (0.15 mmol/L) in methanol was prepared. Different oil concentrations were prepared in methanol and 200 µl of each dilution was mixed with 50 µl of DPPH• solution in a 96-well microtiter plate. The mixture was allowed to stand at room temperature in dark for 30 min. The decrease in absorbance at 517 nm was measured. The radical scavenging activity was measured as a decrease in absorbance of DPPH.

ABTS cation decolorization assay was conducted on various concentrations of Lemon Grass, cinnamon, and clove oil made in methanol. ABTS radical cation was freshly prepared by mixing 14 mM ABTS with equal volume of 4.95 mM potassium persulphate and kept for 24 hours at room temperature. The ABTS radical cation was used for the assay after dilution with Phosphate Buffer Saline (PBS) appropriately. To 50 µl of various concentrations of the oils, 150 µl of ABTS solution was added. After 1 min incubation at room temperature, absorbance was measured at 732 nm. The cation scavenging activity was measured same as with DPPH. The antioxidant activity was calculated as a percentage of inhibition according to the following equation:

$$\% \text{ Radical Inhibition} = \{(\text{Control OD} - \text{Sample OD})/ \text{Control OD}\} \times 100$$

Statistical analysis

Data was analyzed statistically on 'SPSS-16.0' (SPSS Inc., Chicago, II USA) software package as per standard methods (Snedecor and Cochran 1994). Duplicate samples were drawn for each parameter and the whole set of experiment was repeated three times to have total six number of observations (n=6). The mean values were reported along with standard error. The statistical significance was estimated at 5% level ($p<0.05$) and evaluated with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Antimicrobial activity and MIC of essential oils from lemongrass, cinnamon and clove

Antimicrobial activity was performed for lemongrass, cinnamon and clove essential oil by well diffusion method against nine foodborne pathogens. The Results obtained for MIC (ppm) of three oils against the above mentioned organisms is presented in Table 2.

Table 2: Minimal Inhibitory Concentration (ppm) of Lemon Grass, cinnamon and clove essential oils against nine food spoilage microorganisms

Tested Microorganisms	Lemongrass Oil	Cinnamon Oil	Clove oil
Staphylococcus aureus	700	3000	1000
Bacillus cereus	500	1000	3000
Escherichia coli	3000	1000	3000
Shigella flexneri	500	3000	3000
Klebsiella pneumonia	500	3000	3000
Listeria monocytogenes	700	3000	1000
Yersinia enterocolitica	700	3000	3000
Salmonella typhimurium	500	3000	1000
Vibrio parahaemolyticus	700	3000	1000

The MIC value for lemongrass oil ranged from 500-3000 and the maximum activity was observed against Gram Positive organisms as compared to Gram Negative. Mith *et al.* (2014) studied antimicrobial activity of commercial essential oils against food borne pathogens and spoilage organisms. They reported that *Cymbopogon flexuosus* essential oil showed only strong activity against Gram-positive bacteria. The results are in concordance with Naik *et al.* (2010) who also reported higher effectiveness of lemongrass oil in inhibiting Gram positive organisms than Gram negative. MIC value (ppm) of cinnamon oil against the targeted organisms was found in range of 1000-3000. As observed, the cinnamon essential oil was effective against both Gram positive as well as Gram

negative organisms. However, the value for Gram negative organisms was slightly higher than Gram positive. Similar results have been reported by Urbaniak *et al.* (2013) who determined the antibacterial activity of cinnamon bark oil against Gram-positive and Gram-negative isolates belonging to *Staphylococcus, Enterococcus, Enterobacter* and *Acinetobacter* genera come from different clinical specimens. They reported that tested oil was effective in inhibiting all the isolates. Essential oil from clove had MIC values (ppm) ranging from 1000-3000 and was found effective against both Gram positive as well as Gram negative organisms under study. Similar findings have been reported by Fu *et al.* (2007) who tested antimicrobial efficacy of clove essential oil against three Gram positive and Gram negative and two fungi. They reported that MIC of clove oil ranged from 0.062% to 0.500% (v/v) and time kill curves showed clear bactericidal and fungicidal properties. The zones of inhibition (mm) by three essential oils against all the tested microorganisms are depicted in Figs 1-4.

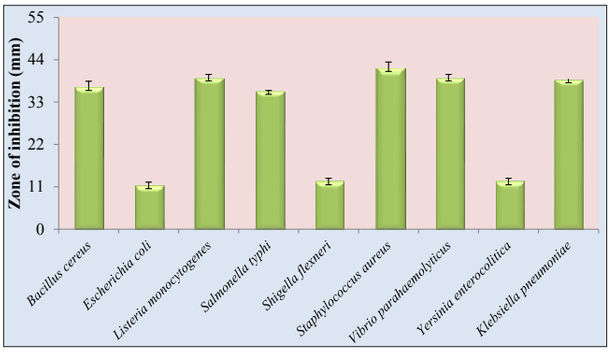

Fig. 1: Zone of Inhibition (mm) of lemongrass oil against common food spoilage and pathogenic microorganisms

The data reveals that a clear demarcated zone, an indicative of inhibition of various microorganisms, was obtained and varied for different essential oils used in study. Lemongrass oil produced zone of maximum diameter for *Staphylococcus aureus* followed by *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *Klebsiella pneumonia* that can be correlated to MIC values wherein strong activity against Gram-positive bacteria was observed. On the other hand, cinnamon oil produced maximum inhibition zone against *Bacillus cereus* and clove oil had highest inhibitory activity for *Salmonella typhimurium*. A good correlation between MIC values and diameter of zone was observed in all the cases.

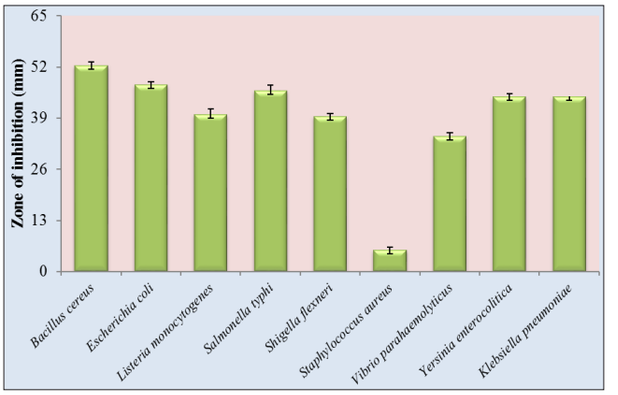

Fig. 2: Zone of Inhibition (mm) of cinnamon oil against common food spoilage and pathogenic microorganisms

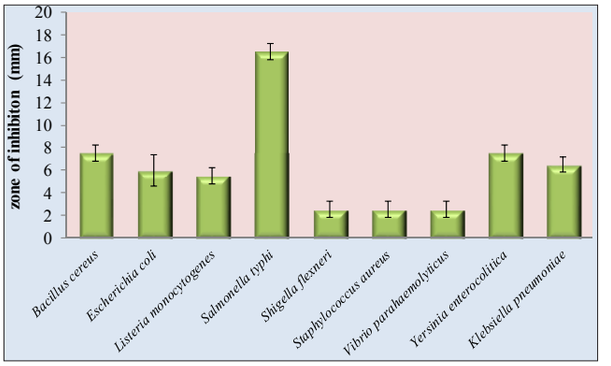

Fig. 3: Zone of Inhibition (mm) of clove oil against common food spoilage and pathogenic microorganisms

Antioxidant efficacy of lemongrass, cinnamon and clove essential oils

The results for antioxidant activity (DPPH and ABTS) of cinnamon, lemongrass and clove essential oils are presented in Tables 3 and 4, respectively. DPPH free radical scavenging activity is due to hydrogen donating ability; the more the number of hydroxyl groups, the higher the possibility of free radical scavenging ability (Chen and Ho 1995). ABTS decolourization assay is an excellent tool for determining antioxidant activity of hydrogen denoting antioxidants. As observed, there was an incremental trend of radical scavenging with increasing concentration of

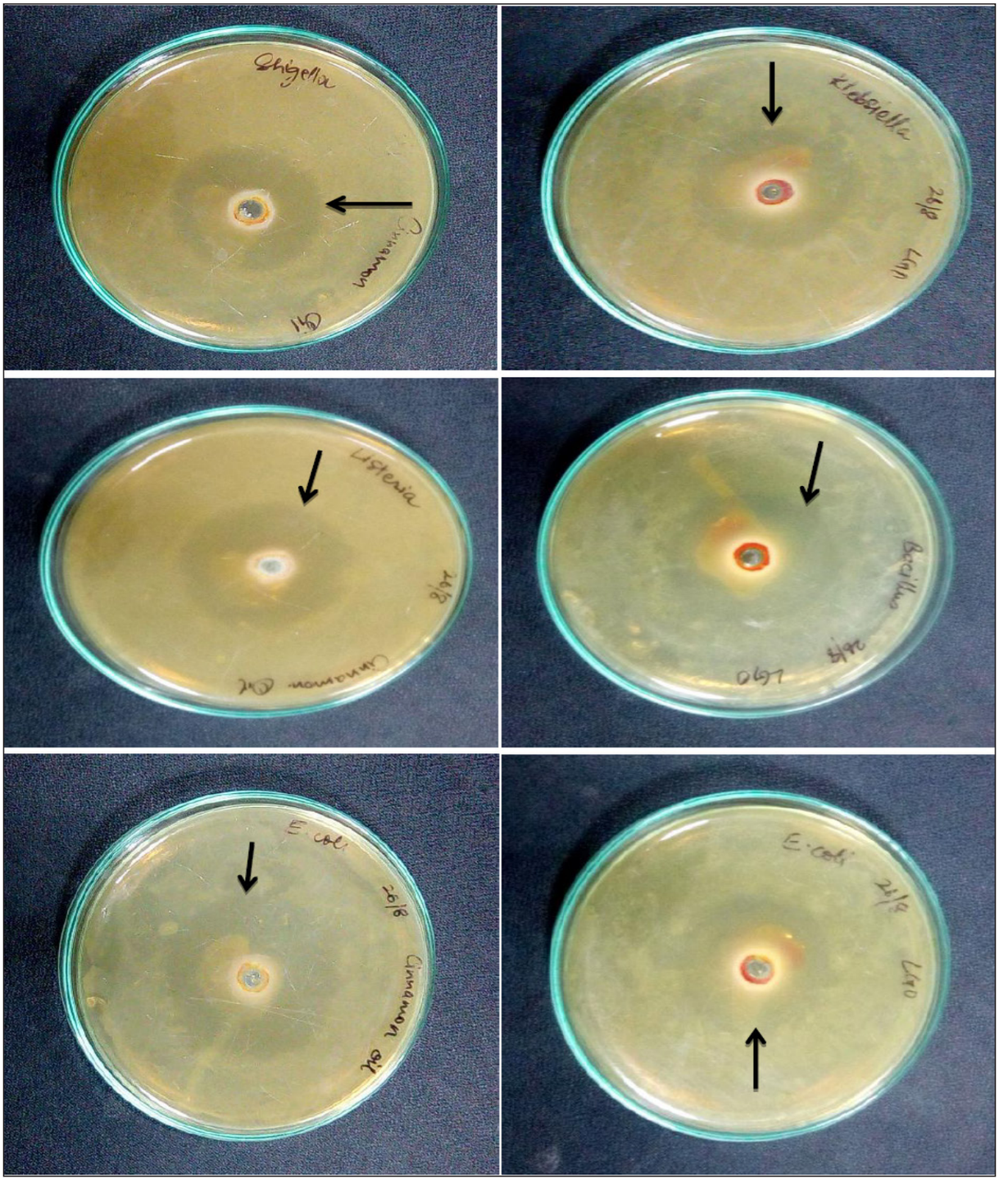

Fig. 4: Zone Of Inhibition Assay Showing antimicrobial activity of Essential oils against common food borne spoilage and pathogenic microorganisms

oil. The higher radical scavenging activity of these oils could be due to presence of active principles i.e. *citral, cinnamaldehye* and *eugenol* in lemongrass, cinnamon and clove oils, respectively.

Table 3: DPPH Radical Scavenging Potential of Lemongrass, cinnamon and Clove essential oils (Mean±S.E.)*

Tested Concentrations (ppm)	Lemongrass Oil	Cinnamon Oil	Clove oil
1000	32.39±1.76^{a}	10.48±0.25^{c}	29.51±1.25^{b}
2000	33.02±0.83^{b}	23.63±0.62^{c}	41.34±0.95^{a}
3000	35.05±0.67^{c}	38.81±0.91^{b}	48.45±0.96^{a}
5000	37.55±1.00^{b}	39.28±0.69^{b}	51.7±1.48^{a}
10000	68.23±1.19^{a}	53.67±1.19^{b}	66.92±1.73^{a}

n=6

*Mean ±S.E. with different superscripts row-wise (a-c) differ significantly (P<0.05)

Table 4: ABTS Radical Scavenging Potential of Lemongrass, cinnamon and Clove essential oils (Mean±S.E.)*

Tested Concentrations (ppm)	Lemongrass Oil	Cinnamon Oil	Clove oil
1000	16.78±1.38^{a}	7.56±0.96^{b}	16.02±1.14^{a}
2000	41.05±1.54^{a}	13.14±0.73^{c}	27.26±0.79^{b}
3000	45.66±1.75^{a}	25.17±0.49^{c}	30.47±1.22^{b}
5000	53.07±1.18^{a}	30.89±0.66^{c}	41.99±0.78^{b}
10000	57.25±1.11^{b}	60.74±0.78^{a}	50.79±1.34^{c}

n=6

*Mean ±S.E. with different superscripts row-wise (a-c) differ significantly (P<0.05)

At 10000 ppm of essential oils concentration lemongrass, cinnamon and clove oil showed 68.23%, 53.67% and 66.92% of DPPH radical scavenging activity, respectively and the value of ABTS radical scavenging at the same concentration was found to be 57.25%, 60.74% and 50.79%, respectively. On the basis of MIC values, the concentration of all three essential oils that was found effective was 0.3% and corresponding concentration was having 35.05%, 38.81% and 48.45% of DPPH radical scavenging activity and 45.66%, 25.17% and 30.47% of ABTS radical scavenging activity for lemongrass, cinnamon and clove oil, respectively.

CONCLUSION

In vitro, antimicrobial and antioxidant study of lemongrass, cinnamon and clove essential oil reveals that they possess potential ability to scavenge free radicals and also has good antimicrobial potential against common food spoilage microorganism having broad spectrum of activity against both Gram positive and Gram negative organisms. These can be used as an alternative to the synthetic additives for control of foodborne pathogens that can minimize the health risk and side effects. Further, they can be tried as potential natural preservatives in foodstuffs, particularly meat products as aromatic and flavoring component without affecting the organoleptic quality and can prevent the oxidative rancidity during storage at refrigeration temperature.

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