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In-vitro Assessment of Antibacterial and Antioxidant Capacity of Essential oils from Cumin (*Cuminum cyminum*) and Lemon (*Citrus limon*) for Future Applications in Meat Industry

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ABSTRACT

Present study was planned to assess *in-vitro* antibacterial and antioxidant activities of cumin and lemon essential oils for future application in food products. *In-vitro* evaluation of antimicrobial activity of both essential oils was done against nine strains of gram-negative and gram-positive microbes. It was determined by Zone of inhibition (ZOI) and minimum inhibitory concentrations (MICs) assays. Results of both oils showed good antibacterial activity against both Gram-negative and Gram-positive bacteria. The MIC values ranged from 2000 to 15000 ppm for cumin oil, whereas it ranged from 6000 to 15000 ppm for lemon essential oil. The antioxidant and antiradical scavenging activity of the both oils were determined by means of DPPH and ABTS assay. Examined essential oils showed a free radical scavenging activity, ranging from 19.31 to 92.41% of DPPH inhibition and 10.32 to 76.78% for ABTS assay for cumin oil and 8.63 to 66.03% of DPPH inhibition and 8.14 to 63.88% for ABTS assay for lemon essential oil. It was observed that cumin essential oil exhibited better antioxidant capacity in terms of free radical inhibition as compared to lemon essential oil. It can be concluded that both cumin and lemon essential oils possess strong antibacterial as well as antioxidant potential for applications as natural preservatives in meat and other food industries.

HIGHLIGHTS

- Antimicrobial and antioxidant efficacy of cumin and lemon essential oils was investigated.
- The results are promising and strengthen the candidature of these essential oil for future application in food products as natural preservatives.

Keywords: Cumin essential oil, Lemon essential oil, ZOI, MIC, DPPH, ABTS, Meat

Meat and meat products are one of the most acceptable food products world-wide and play an important role in human diet being a source of low-cost quality animal protein with high biological value and other essential nutrients (Mehta *et al*., 2013; Mehta *et al*., 2015). Meanwhile it also provides an ideal environment for growth of food microbes and lipid oxidation which ultimately leads to decrease in the quality. An increase in lipid oxidation due to microbial growth affects flavor as well as pose threat to human health (Sallam *et al*., 2004). So, now a days consumers are getting aware about healthier food products and they demand for alternate natural additives as compared to synthetic or chemical food preservatives (Goswami *et al*., 2020), which are associated with damage to consumer health and also leads to various health conditions and economic loss (Jayasenaand Jo, 2013).

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Various spices, plant extracts and essential oils hold potential as natural additives in perishable meat products owing to superior antimicrobial and antioxidant actions. Further, their constituents have been proved to have protective action, when consumed. Almost 3000 essential oils have been identified and out of them, 300 are commercially available in the market (Burt, 2004). During the nineteenth century, essential oils were predominantly used in flavour, perfumery, and cosmetics but their pharmacological uses like antibacterial, antifungal, anti-inflammatory, antioxidant, astringent, etc. were studied simultaneously. The prominent antioxidant activity of these essential oils is primarily due to phenolic components, flavonoids, alkaloids, tannins, etc. Further, they also contain various antimicrobial components such as geraniol, menthol, cinnamyl alcohol, linalool, citronellal, carvacrol, cuminaldehyde, cinnamaldehyde, eugenol, thymol, estragole, carvone, chavicol etc. depending upon the type of oil. Thus they are seen as potent candidate for increasing shelf life of different foods such as fruits, vegetables and meat products. In addition, most of them are classified Generally Recognized as Safe (GRAS) by USDA.

Cumin essential oil has a special hallowed position in our traditional medicine for its beneficial effect on general health as well as due to its antioxidant and antispasmodic properties (Niaki *et al*., 2016). It is derived from the dried seed of the herb, *Cuminum cyminum*, a member of the Parsley family having cuminaldehyde as a principal active constituent (Srinivasan, 2018), possessing higher antioxidant and antimicrobial action. Black cumin is a cultigen that has been known since time immemorial. Antioxidant activity of cumin oil is due to monoterpene alcohols, flavonoids, and other polyphenolic molecules. It also has antimicrobial activity against several microorganisms like *Klebsiella pneumonia, Streptococcus mutans* and *Streptococcus pyogenes* (Singh *et al*., 2017). Higher concentration of *p*-coumaric acid from cumin seed oil is known to be the bioactive compound responsible for both antibacterial and antioxidant activities. Lemon essential oil extracted from *Citrus limon* has also a unique place in Ayurveda for its medicinal properties. It is majorly produced in India and other tropical and subtropical regions of Asia. It is a good source of flavonoids, limonoids, coumarins polyphenols, sterols, volatile oils, organic acids, and furanocoumarins, and several valuable bioactive compounds, the most valuable one is antioxidant in nature. Further, it possesses potential antiviral, antimicrobial, antifungal, anticancer, anti-inflammatory, insecticidal, hypoglycaemic and antitumor activity (Saeb *et al*., 2016). The application of these essential oils is a relatively novel area for perishable commodities like meat and meat products. The present study is planned to evaluate *in vitro* antioxidant and antimicrobial efficacy of cumin and lemon essential oils as prospective natural preservative for application in meat industry by substituting synthetic additives.

MATERIALS AND METHODS

Cumin and lemon essential oils: Source and composition

Cumin essential oil (CEO) and Lemon essential oil (LEO) were purchased from the Kanta Enterprises Private Limited, Noida, Uttar Pradesh, India. The colour of LEO was pale yellow with lemon rind odour, while CEO was comparatively dark yellow in appearance with spicy sweet odour. At 20 □ refractive index of LEO was between 1.494 to 1.506 and CEO was around 1.494 to 1.506, while specific gravity of LEO was in range 0.905 to 0.925 and CEO was within 1.449-1.467. The detailed composition of both essential oils as per GC-MS analysis is provided under Table 1.

Bacterial strains and growth conditions

Pure freeze-dried microbial cultures were purchased from Institute of Microbial Technology (IMTECH), Chandigarh, India and nine organisms viz., *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 723), *Listeria monocytogenes* (MTCC 1143), *Pseudomonas aeruginosa* (MTCC 741), *Salmonella enterica* serovar *Typhi* (MTCC 733), *Shigella flexneri* (MTCC 1457), *Bacillus cereus* (MTCC 1272), *Yersinia enterocolitica* (MTCC 840), and *Vibrio* (MTCC 7030). These cultures were revived and stock cultures were being prepared and maintained at -20°C by regular passaging.

Antimicrobial activity estimation: determination of zone of inhibition (ZOI) and Minimal inhibition concentration (MIC)

Antimicrobial activity of both oils was estimated as per modified agar well diffusion method (Bag and

Table 1: Chemical composition (%) of Cumin and Lemon essential oil (GC-MS analysis)

Sl. No.	Cumin Essential Oil		Lemon Essential Oil	
	Name of active compound	**% age**	**Name of active compound**	**age%**
1	*α*-Pinene	1.09	*α*-Pinene	3.03
2	p-Cymene	14.96	*β*-Pinene	10.60
3	*α*-Phellandrene	0.48	D-Limonene	53.18
4	*β*-Pinene	17.57	p-Cymene	2.28
5	*γ*-Terpinene	20.49	Decanal	1.03
6	p-Menthe-1,3-Dien-7-al, p-Menthe-1,4-Dien-7-al	6.70	*α* –Terpineol	6.88
7	Cuminal	26.90	Linalool	3.29
8	*β*-Myrcene	0.77	*γ*-terpineol	1.19
9	Carvacol	0.13	Citral	2.91
10	Miscellaneous minor constituents	11.04	Geraniol	1.26

Chattopadhyay, 2015). Briefly, Microbial cultures were re-energized in sterile BHI broth by incubating overnight for 37°C. Later, the density of each bacterial working inoculum was adjusted equal to 5×10^5 CFU/ml. BHI agar plates were prepared and kept undisturbed for 24 hours. 100µl of each inoculum was spread uniformly with a glass rod spreader on nutrient agar and well of 10 mm diameter were bored using sterile cork borer. 100 µl of both oils was poured in each well and, plates were pre-incubated at refrigeration temperature (4±1°C for 1 hour) for quick diffusion of oil afterwards overnight incubated at 37°C. Results of Zone of Inhibition (ZoI) were determined by using zone scale (Hi-Media) and expressed in millimetre (mm). All the tests were performed in duplicate, and the mean values of the diameter of inhibition zones were recorded.

The minimal inhibition concentration values of both essential oils were estimated against above mentioned bacterial cultures using the method described by Punya *et al*. (2019). Microbial cultures were incubated at 37°C for 12 h and density of suspension was adjusted to 0.5 McFarland standard. Both the active oils were dissolved in 10% dimethylsulfoxide (DMSO) and then serial two-fold dilutions were made in a concentration between 0.025% to 3.75% using nutrient broth. 100 µl essential oils were added in flat-bottom 96-well micro-titre plates with 100 µl nutrient broth in each well. Each solution was thoroughly mixed using micro-pipette and incubated at 37°C for 24 hours. After incubation, 100 µl of each sample was taken from the respective wells and spread over nutrient agar plates to check the visible growth of bacteria on overnight incubation.

Antioxidant activity of cumin and lemon essential oil

The antioxidant activity of both essential oils was checked spectrophotometrically in terms of radical scavenging activity or hydrogen donating ability using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2, 2′-azinobis3-ethylbenzthiazoline-6-sulphonate (ABTS) radical. Ability of essential oil to donate hydrogen atoms or electrons was measured from bleaching of coloured methanoloic solutions.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Radical scavenging potential of essential oils 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. Briefly, a solution of DPPH (0.15 mmol/L) in methanol was prepared. Oil concentrations ranging 100 to 20000 ppm were prepared in methanol and 200 µl of each dilution was mixed with 50 µl of DPPH solution in a 96-well microtiter plate, achieving a final volume of 3.0 ml. After that solution was placed in incubation in dark room at room temperature for 30 min and the absorbance was measured at 517 nm. DPPH radical scavenging capacity results were measured in percentage and calculated as per the following equation:

Scavenging effect (%) =

$$\left[1-\frac{\text{O.D. Sample} - \text{O.D. Blank}}{\text{O.D. Control}}\right]\times 100$$

2-2-azinobis-3ethylbenthiazoline-6-sulphonic acid radical activity (ABTS)

With slight modifications, antioxidant potential of cumin and lemon essential oil was determined using 2, 2′-azinobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) cation decolourisation assay as per method followed by Kaur *et al*. (2020). Primarily solution containing ABTS˚ radical (7 mM) and potassium persulfate (2.45 mM) (1:0.5) was kept in the dark room for 18 h at room temperature. Aliquot of solution was diluted with methanol and set to 0.70 ± 0.03 optical density for ABTS working solution. ABTS working solution (150 mL) was added to 50 mL of various concentrations (100 to 20000 ppm) of both essential oils separately. After a 1-min incubation at room, the optical density was measured at 732 nm.

Scavenging effect (%) =

$$\left[1-\frac{\text{O.D. Sample} - \text{O.D. Blank}}{\text{O.D. Control}}\right]\times 100$$

The experiment was performed in triplicate and Butylated hydroxyltoluene (BHT) was used as positive control in both above mentioned antioxidant assays.

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). The whole experiment was repeated three time and analyzed in duplicate and results were expressed as Mean± SE.

RESULTS AND DISCUSSION

Biological activity of essential oils is largely dependent upon its chemical composition and percentage of active component. Further, it is affected by region, surrounding environment and time of harvesting of plant from which it is extracted. The chemical composition as per the certificate of analysis (GC-MS) provided by the manufacturer revealed that cuminal (26.90%) and D-Limonene (53.18%) were the principal active components in cumin and lemon essential oil, respectively. Presence of β–pinene (10.60%) in lemon oil results in typical aroma which quite different from other oils. Singh *et al.* (2014) reported p-cymene (31.4%) and thymoquinone (37.6%) as the major constitutes of black cumin essential oil, whereas Farahmandfar *et al.* (2018) and Hsouna *et al.* (2017) observed that *Limonene* was the major constituent in lemon essential oil tested by them. All these differences in composition might be attributed to the regional disparities and time of harvesting of plants.

Antimicrobial efficacy of cumin and lemon essential oils

Essential oils are complex combination of a various constituents which are already known for their antimicrobial properties. The antimicrobial action of essential oils can be due to combination of more than one active components and sometimes these components may act synergistically producing better actions as compared to individual components. Gupta *et al.* (2017) also observed that *in-vitro* antimicrobial effect of essential oils was different due to their chemical composition and specific microorganism tested. Many researchers showed their promising antimicrobial activity against a number of food-borne pathogens and spoilage bacteria and at the same time they were also showing potential antioxidant activity. In the present study, antimicrobial efficacy of both essential oils i.e. cumin and lemon was determined by Zone of inhibition and minimal inhibitory concentration (MIC) against different food spoilage organisms. The well size of 10 mm in diameter was taken for the experiment and results are expressed in table 2 and Fig. 1 and 2.

MIC (ppm) of cumin essential oil against targeted organisms was found in the range of 2000-15000. The cumin oil exerted a considerable inhibitory effect against all the organisms except *P. aeruginosa* which was depicting highest MIC (15000 ppm). In general, gram negative bacteria had higher resistance to cumin essential oil than gram positive which might be due to complex lipopolysaccharide-based membrane structure in former that might have inhibited diffusion of hydrophobic essential oil, resulting in lesser cell death. Similar findings have been reported by Behbahani *et al.* (2020) who

reported higher inhibition for gram positive organisms on exposure to cumin essential oil. Takma and Korel (2019) also reported an effective antimicrobial action of black cumin oil against *Staphylococcus aureus* and *Escherichia coli* when incorporated in active packaging films. The results of zone inhibition assay, as depicted in Fig. 1 revealed that the maximum inhibition diameter was observed for *Salmonella typhimurium* (30±0.89) and *Pseudomonas aeruginosa* (30±0.86) followed by *Vibrio parahaemolyticus* (28±0.45), whereas minimum zone diameter was observed for *E. coli* (18±0.86). Similar pattern of results has been demonstrated by Wanner *et al*. (2010) and Purkait *et al*. (2018) who also reported that cumin essential oil was found highly effective against all tested food microbes, both gram positive and gram negative.

Lemon essential oil was also found to have potential antimicrobial effect when tested *in-vitro* against food spoilage and pathogenic microorganisms. The results obtained for MIC (ppm) of lemon oil against the food borne pathogenic organisms is presented in Table 2. The MIC value ranged from 6000-15000 ppm and was effective against both Gram Positive and Gram-Negative organisms. The zone of inhibition observed against organisms reveal that maximum diameter was observed for *Salmonella typhimurium* (28±0.74) followed by *Shigella flexineri* (26±1.21). Similar results for antimicrobial activity has been reported by Gupta *et al.* (2017) who compared the antimicrobial activities of lemon oil and extract against common food borne pathogens.

Table 2: Minimal Inhibitory Concentration (ppm) of cumin and lemon essential oil against nine food spoilage microorganisms

Sl. No.	Test Microorganisms	MIC of cumin essential oil (ppm)	MIC of Lemon essential oil (ppm)
1	*E. coli*	2000	6000
2	*P. aeruginosa*	15000	15000
3	*V. parahaemolyticus*	3000	13000
4	*Y. enterocolitica*	3000	6000
5	*S. typhimurium*	2000	6000
6	*S. flexneri*	3000	6000
7	*L. monocytogenes*	2000	11000
8	*B. cereus*	2000	11000
9	*S. aureus*	3000	6000

They found that *Staphylococcus aureus* was highly sensitive to the lemon oil presenting lowest MIC of 6.25 mg/mL followed by *Bacillus cereus* (MIC= 12.5 mg/mL). Antibacterial activity of pectin based edible films incorporated with Mexican lime essential oil was evaluated by Aldana *et al.* (2015) and they found that it was effective against *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes,* however highest inhibitory activity was reported against *E. coli.* In

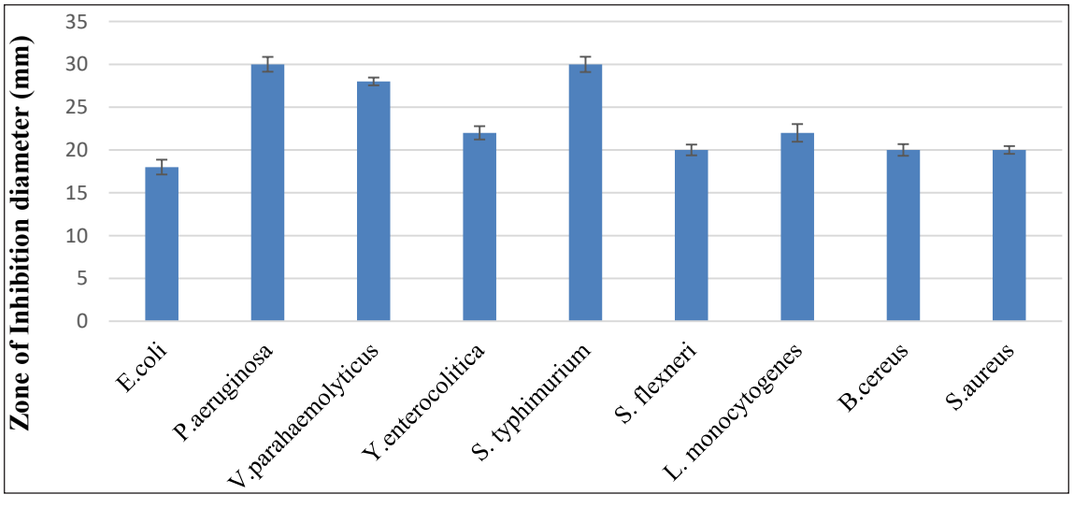

Fig. 1: Zone of Inhibition Assay (mm) of cumin essential oil against nine food spoilage microorganisms (Mean± S.E.)

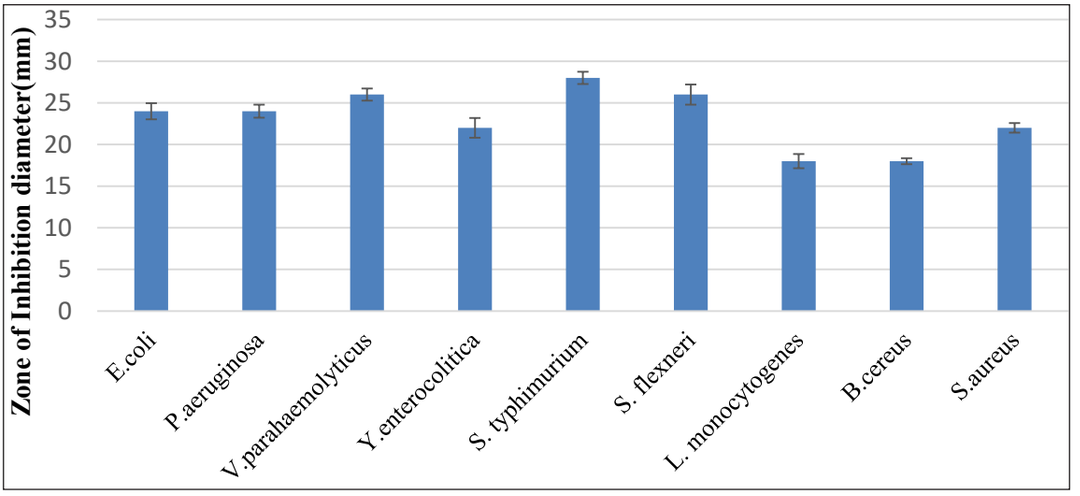

Fig. 2: Zone of Inhibition Assay (mm) of lemon essential oil against nine common food spoilage microorganisms (Mean± S.E.)

general, both cumin and lemon essential oils showed significantly strong antimicrobial activity against all tested gram-positive and gram-negative microbes but CEO showed slightly stronger antimicrobial effect as compared to LEO. This could be attributed to active antimicrobial constituents of cumin essential oil like, myrcene, p-cymene, longifolene which were responsible for reducing bacterial growth (Belal *et al*., 2017; Singh *et al*., 2014), while lemon essential oil had limonene and limonene hydroperoxide as a major active antimicrobial component (Ozogul *et al*., 2015).

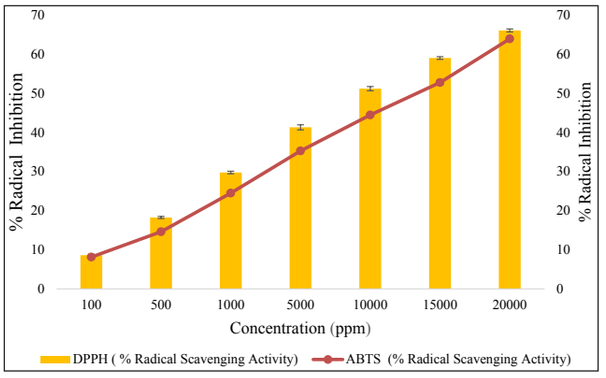

Fig. 3: DPPH and ABTS radical scavenging potential of cumin essential oil (Mean± S.E.)

Antioxidant efficacy of cumin and lemon essential oils

In vitro antioxidant assays mimic the oxidation-reduction pathways commonly occurring in biological systems and are helpful in estimating antioxidant potential of various biomolecules (Punya *et al*., 2019). The results for radical scavenging activity as depicted by DPPH and ABTS estimation of both cumin and lemon essential oils are presented in Fig. 3 and 4. A concentration-dependent antioxidant potential was observed for both the essential oils. There was an incremental trend of radical scavenging with increasing concentration of oil for DPPH and similar observations were reported for ABTS value.

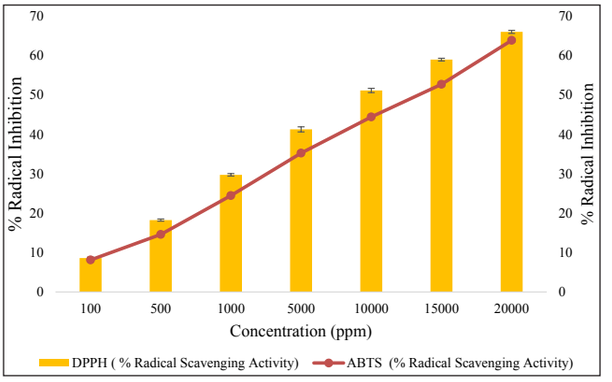

Fig. 4: DPPH and ABTS radical scavenging potential of lemon essential oil (Mean± S.E.)

Percent inhibition by DPPH assay was found in range of 19.31 % to 92.41 % in cumin essential oil and 8.63% to 63.0% in lemon essential oil. Although ABTS radical

assay displayed similar results when compared with DPPH radical scavenging assay, but slightly lower values were reported ranging from 10.32% to 76.78% in cumin essential oil and 8.14% to 63.88% in lemon essential oil at tested concentrations. The higher radical scavenging activity of these oils could be due to presence of active principles i.e. *Cuminal* and D-*Limonene* in cumin and lemon oil, respectively. At 20,000 ppm concentration, cumin and lemon oil showed 92.41% and 66.03% of DPPH, and 76.68 % and 63.88 % of ABTS radical scavenging activity, respectively. On the basis of MIC values, the concentration of cumin and lemon oil (1.5%) was found effective and corresponding concentration was having 79.04% and 58.99% of DPPH radical scavenging activity and 67.83% and 52.72% of ABTS radical scavenging activity for cumin and lemon oil, respectively. On comparative analysis, cumin essential oil had better antioxidant potential as compared to lemon essential oil at all the tested concentrations.

CONCLUSION

In-vitro analysis of antimicrobial and antioxidant potential of cumin and lemon essential oils revealed that both the oils exhibit superior actions against all the tested gram positive and gram-negative organisms. The activity may be attributed to the active constituents along with the possible synergism between them. A higher antimicrobial effect against common food spoilage and pathogenic microorganisms with a broad spectrum of activity against both Gram positive and Gram-negative organisms in addition to significant radical scavenging activity makes them an ideal candidate for application in meat industry. It can be concluded that both these essential oils can serve as a key replacer of synthetic antimicrobials and antioxidants in meat and food industries, thereby minimizing adverse effects on human health Comparatively, cumin essential oil can be a choice for application due to superior actions as compared to lemon essential oil.

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