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Vivek Shukla
Assistant Professor, Department of Livestock Products Technology, Mumbai Veterinary College, Parel, Mumbai, Maharashtra, India

SK Mendiratta
Division of Livestock Products Technology, ICAR-IVRI, Izatnagar, Uttar Pradesh, India

RJ Zende
Department of Veterinary Public Health, Mumbai Veterinary College, Parel, Mumbai, Maharashtra, India

Shekhar Badhe
Department of Livestock Products Technology, Mumbai Veterinary College, Parel, Mumbai, Maharashtra, India

Corresponding Author
Vivek Shukla
Assistant Professor, Department of Livestock Products Technology, Mumbai Veterinary College, Parel, Mumbai, Maharashtra, India

Evaluation of the *in-vitro* antimicrobial activity of clove and oregano essential oils against foodborne microbes and its comparison with antibiotics

Vivek Shukla, SK Mendiratta, RJ Zende and Shekhar Badhe

Abstract
The growing concern about the harmful effects of synthetic preservatives and rising stringency on the use of natural preservatives, is compelling the scientist to search for natural methods of preservation of meat products. The consumers are desperate for products preserved by natural preservatives with effective antimicrobial characteristics against food borne microbes. The present study evaluated the *in vitro*-antimicrobial efficacy of clove and oregano essential oils against selected foodborne microbes and their comparison against selected antibiotics. GC-MS analysis of essential oils revealed the carvacrol and eugenol as the principal component of the oregano and clove essential oil, respectively. The *in-vitro* antimicrobial activity of essential oils and their comparison with antibiotics, MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration), revealed that oregano essential oil had significantly higher ($P<0.05$) antimicrobial activity than clove essential oil and was as effective as antibiotics against selected foodborne microorganisms. *Pseudomonas aeruginosa* was the most resistant to essential oils among the selected microbes, whereas clove essential oil was utterly ineffective against it. The results indicated that both essential oils have colossal scope for utilization as natural preservative for meat and meat products.

Keywords: Essential oils, foodborne microorganisms, natural preservation, MIC, MBC

Introduction
The use of synthetic preservatives has a negative perception among the consumers, and using natural preservatives for meat and meat products dodging synthetic preservatives is considered as a remarkable development (Marques *et al.*, 2019) [1]. Natural preservatives in spices and condiments are used for centuries in various cuisines worldwide (Maria *et al.*, 2016) [2]. Besides providing flavour and taste in the meat products, they have a unique ability to inhibit food pathogens and spoilage microorganisms (Bhavaniramya *et al.*, 2019) [3]. Essential oils, which form the active principle of spices, have been used to enhance shelf-life and microbial inhibition in various meat (Radhakrishnan *et al.*, 2014; Mupalla *et al.*, 2014, Petrou *et al.*, 2012) [4, 6] and meat products (Sojic *et al.*, 2019; Pabast *et al.*, 2018; Peasvento *et al.*, 2015; Radunaj *et al.*, 2019; Nikmaram *et al.*, 2018) [7, 8] without affecting sensory characteristics.
Plants synthesize essential oils to protect themselves from pathogens, but they also possess medicinal properties which benefit human health (Patel and Gogna, 2015; Zuzarte and L. Salgueiro, 2015; Islam *et al.*, 2016; Lenard*ao et al.*, 2016) [12, 13, 14, 15]. The essential oils are a complex mixture of several volatile components obtained from various aromatic plants (Dima and Dima, 2015), and antimicrobial activity is mainly attributed to phenolic compounds (Pesavento *et al.*, 2015) [9] such as carvacrol and thymol. Several spices are known for their essential oil content and biological activity, and oregano and clove essential oils are among the most commonly used essential oils that form an essential part of the spice mixture used in various cuisines worldwide. Several reports have confirmed antibacterial and antioxidant activities of these essential oils and their components (Mith *et al.*, 2014; Radhakrishnan *et al.*, 2014; Pinheiro *et al.*, 2015 Sokovic *et al.*, 2010; Bassanetti *et al.*, 2016) [17, 4, 18, 19, 20].
The composition and percentage of the active component as well as the efficacy of essential oils vary with several factors such as species, subspecies or variety of plants (. Sarac and Ugur, 2008) [21], geographical locations (Sarac and Ugur 2008; Mechergui *et al.*, 2010) [21, 22], drying methods (Di Cesare *et al*., 2003) [23] extraction methods (Burt, 2004; Karakaya *et al.*, 2011) [24, 25]. However, determination of in-vitro antimicrobial activity provides a preliminary idea of essential oil and its intended use in the food system and its use as natural preservatives.

Therefore, the present study was planned to estimate the in-vitro antimicrobial activity of clove and oregano essential oils and compare their antimicrobial efficacy with antibiotics against foodborne microbes.

Materials and Methods

Essential oils, Antibiotic discs, Chemicals, Broth and Culture Media

Clove and oregano essential oils were purchased from Synthite Industries (Kerala, India) and stored at 4°C before use. Antibiotic discs *viz*. chloramphenicol (30mcg), streptomycin (10mcg), ampicillin (10mcg), oxytetracycline (30mcg) and ceftriaxone (30mcg) of 6 mm size were procured from HiMedia®, India. Sterile discs were also procured from HiMedia®.All Chemicals, broth and culture media used were of analytical grade and procured from HiMedia®, India.

GC-MS analysis of EOs

The GC-MS analysis of EOs was performed using Agilent-6890 N gas chromatograph with an HP 5973 mass spectrometer detector using HP-5Ms capillary column (30.0 m × 0.25 mm × 0.25 µm). Helium (99.999%) was used as the carrier gas with a flow rate of 1.0 ml/min, the injection volume was 1µl, and the split ratio was 1:1. Initially, the temperature was maintained at 100°C for 3 min, then raised to 250°C in steps of 20°C min^{-1}. Compounds in the extracts were identified by matching their mass spectra and retention times with pure compounds.

Procurement Bacterial Strainsand preparation of stock culture

The microbial cultures were procured from The Microbial Type Culture Collection and Gene Bank, Chandigarh, Punjab. For assessing the antibacterial properties of essential oils, four strains of foodborne microbes were used, *viz*. *Salmonella* Typhimurium MTCC 98, *Staphylococcus aureus* MTCC 902, *Pseudomonas aeruginosa* MTCC 2453 and *E. coli MTCC* 723. Colonies of the isolates were inoculated in tryptone soybroth and incubated at 37 ºC for 24 hrs. After incubation, the culture suspension was poured into sterile centrifuge tubes and centrifuged at 5,000rpm for 10 min (Remi, Model R-2912M). After centrifugation, the supernatant was discarded, and the pellet was re-suspended in10 ml of sterile phosphate buffer and centrifuged again as previously described. The final supernatant was discarded, and the pellet was re-suspended in 1 mL of tryptone soybroth with 30% glycerol lsolution in a 2 ml cryovial. Stock cultures were stored at -45ºC until ready for use.

Comparison of*in-vitro* antimicrobial activity of EOs with antibiotics

The procedure of Mith *et al.* (2014) [17] was used tocompare the *in-vitro* antimicrobial activity of EOs with antibiotics. Disc diffusion method (zone of inhibition/agar diffusion assay) is a direct contact method used to measure antimicrobial activity. For the disc diffusion test, 100µl of one-day-old bacterial suspension from each Brain Heart Infusion (BHI) broth inoculated with different microorganisms containing a bacterial concentration of 10^6CFU/ml was poured over Mueller Hinton (MH) agar plates, and uniform spreading was done using L-spreader. For comparison with antibiotics, antibiotics discs were placed in the corners of the petri dish while plain sterile discs of 6mm size incorporated with 1µl of essential oil were placed in the centre. After 24-48hrs of incubation of plates at 37ºC under aerobic conditions, the visible zone of inhibition around each disc (colony free perimeter) was measured with Vernier caliper.

Determination of minimum inhibitory concentration (MIC) of selected EOs

Determination of MIC of selected EOs by disc diffusion method

For the determination of MIC by the disc diffusion method, the procedure of Sharma *et al*. (2017) [26] was followed with suitable modifications. For the disc dilution method, sterile 6mm discs (HiMedia®, Mumbai, India) with different dilutions of each essential oil were deposited over Mueller Hinton (MH) agar plates inoculated with 100µl of one-day-old BHI broth having a bacterial count of 10^6CFU/ml. For clove essential oil, the volume deposited per disc varied from 0.125 to 10.0µl, while for oregano essential oil, the volume varied from 0.015625 to 8.0 µl. After 24-48 hours of incubation of plates at 37ºC under aerobic conditions, the visible zone of inhibition around each disc (colony free perimeter) was measured with Vernier caliper. The disc with the highest dilution of EOs showing a visible zone of inhibition was considered as MIC of essential oil against the test organism.

Determination of MIC of selected EOs by Macro-tube dilution method

For the determination of Minimum Inhibitory Concentration (MIC) of clove and oregano essential oil against selected foodborne pathogens by Macro-tube dilution method, the procedure of Mith *et al.* (2014) [17] was followed with modifications. 5.0ml of BHI broth was added to sterile culture tubes, and an increasing volume of essential oils was added to each tube. For clove essential oil, the volume added per tube varied from 0.25 to 2.5µl, while in the case of oregano essential oil, it varied from 0.25 to 10 µl. After that, 100µl of the diluted microbial suspension having abacterial count of 10^6CFU/ml was inoculated into each sterile tube containing 5ml of BHI broth and incubated overnight at 37ºC. The tube with the minimum volume of essential oil showing an absence of turbidity after overnight incubation was considered MIC.

Determination of Minimum Bactericidal Concentration (MBC) of selected EOs

The method of Mith *et al.* (2014) [17] was followed for the determination of minimum bactericidal concentration. The MBC was determined by subculturing 100µl from each negative culture tube onto Plate Count Agar (PCA) plates. MBC was defined as the lowest concentration resulting in a negative subculture or giving the presence of only one colony after incubation.

Statistical analysis

All experiments were replicated thrice in duplicate (6 determinations in total per test condition). For statistical analysis purposes, the value of the six determinations was used per sample such that the statistics describe the variation between samples with n=6. The data generated were compiled and analyzed using SPSS (Statistical Package for Social Sciences, version 26.0 for Windows; SPSS, Chicago, IL, USA) with randomized block design and subjected to analysis of variance. Duncan's multiple range test (27) was carried out for comparing means. The smallest difference (D_5%) for the

two means to be significantly different (p<0.05) was reported.

Results and Discussions

GC-MS analysis of clove and oregano essential oils

The results for the GC-MS analysis of clove and oregano essential oils are presented in Table 1. The results revealed carvacrol and eugenol as the principal component in oregano and clove essential oil are carvacrol (64.90%) and eugenol (75.17%) in oregano and clove essential oil, respectively. Similarly, carvacrol and eugenol were the principal ingredients in oregano and clove essential oil, respectively, with a concentration of 46.37% of carvacrol and 84.75% of eugenol. However, the varied concentration of carvacrol and thymol, respectively, in oregano essential oil and eugenol in clove essential oil has been reported in different studies (Radhakrishnan *et al.*, 2014) [4]. This disparity in the concentration of active components could be attributed to the various factors *viz*. species, subspecies or variety of plants (Sarac and Ugur, 2008) [21], geographical locations (Mechergui *et al.*, 2010) [22], harvesting seasons (Donaldson *et al*, 2005) [28], drying methods (Di Cesare *et al.*, 2003) [23], and extraction methods (Karakaya *et al.*, 2011) [25].

Table 1: The composition of oregano and clove essential oil

Sr. No.	Oregano essential oil		Clove essential oil	
1	Component	Percent (%)	Component	Percent (%)
2	Carvacrol	64.90	Eugenol	75.17
3	Linalool	10.80	Beta Caryophyllene	11.57
4	M- Cymene	5.80	Eugenyl Acetate	6.14
5	Gamma terpinene	2.50	Humulene	1.38
6	Thymol	2.10	Minor components	5.74
7	Beta Caryophyllene	1.90		
8	Terpinene- 4-ol	1.68		
9	Minor components	10.32		

n=6, Expressed as percentage of the total peak area.

Evaluation of the *in-vitro* antimicrobial activity of EOs and comparison with antibiotics

The Mean±S.E. of the diameter of zone of inhibition against selected foodborne microorganisms created by different antibiotics and essential oils are presented in Figure 1. Oregano EO produced a significantly larger (p<0.05) zone of inhibition against *E. coli* as compared to clove essential oil and all other antibiotics. Even the diameter of the zone of inhibition produced by 4thgeneration antibiotics, i.e. ceftriaxone, was significantly smaller (p<0.05) than oregano EO. The diameters of the zone of inhibition of chloramphenicol, streptomycin, ampicillin and oxy-tetracycline against *E. coli* were significantly smaller (p<0.05) than oregano EO. The clove essential oil also produced a significantly larger (p<0.05) zone of inhibition than streptomycin and ampicillin, but no significant (p>0.05) difference was found between oxytetracycline and clove essential oil.

The diameters of the zone of inhibition produced by antibiotics and essential oils against *Staphylococcus aureus* was significantly (p<0.01) different from each other. Critical difference analysis revealed that the diameter of the zone of inhibition created by ampicillin was significantly (p<0.05) higher than ceftriaxone, oregano and clove essential oils. Clove oil was the least effective against *Staphylococcus aureus* among all the antibiotics and essential oils. The results revealed that oregano essential oil was significantly (p<0.05) more effective than clove essential oil against *Staphylococcus aureus*. However, both of them were significantly (p<0.05) less effective than ampicillin and ceftriaxone. *Salmonella* Typhimurium was the most sensitive to ceftriaxone among all the antibiotics and essential oils, followed by oregano essential oil. In contrast, clove essential oil was the least effective among all the antibiotics and essential oils against it.

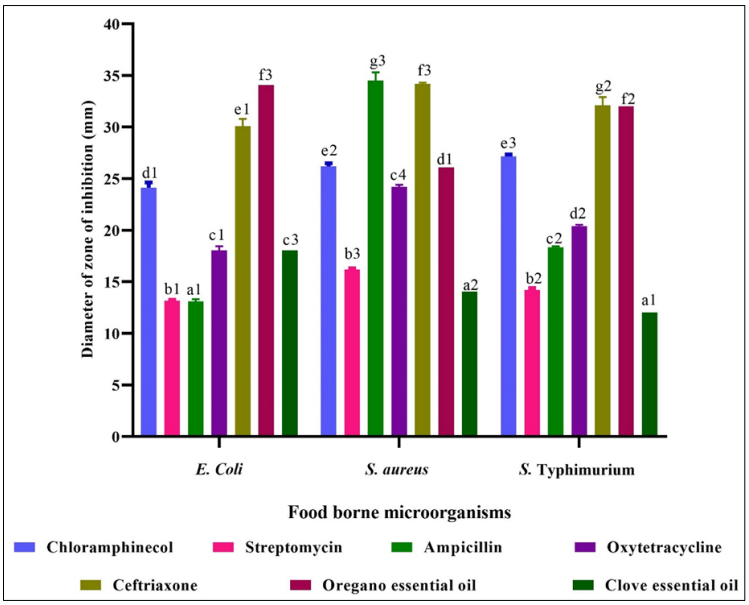

Fig 1: *In-vitro* antimicrobial activity of essential oils against foodborne microorganisms and their comparison with antibiotics (Mean±S.E.); n=6; Mean±S.E. with different superscripts differ significantly (p<0.05)

The results indicated that the antimicrobial efficacy of essential oils was comparable to that of antibiotics against foodborne microorganisms, where oregano essential oil was more effective than clove essential oil. It has been reported that oregano essential oil was more effective against *E. coli* O157: H7 than clove essential oil (Mith *et al.,* 2014) [17]. Further, it has also been reported that oregano EO and its active components, i.e. thymol and carvacrol, exhibited the highest efficacy against *Staphylococcus aureus* among different essential oils, which corroborated with the findings in the present study (Hussain *et al.,* 2008) [29]. The results were in accordance with the GC-MS analysis of the oregano and clove EOs, where carvacrol concentration was 64.90% compared to clove EO, where eugenol was present in a concentration of 75.17%.

The increased antimicrobial activityof oregano essential oil could be sttributed to the phenolic compounds having oxygenated terpenes *viz*. carvacrol and thymol (Sokovic *et al*., 2010; Castilho *et al.,* 2010) [19, 30]. In contrast, eugenol, an oxygenated molecule but not derived from terpene, has lesser antimicrobial activity (Clarke, 2008) [31]**.** Similar results against *Listeria monocytogenes*, *Salmonella* Typhimurium, *E. coli* and *Bacillus thermosphacta* have been observed where inhibitory diameter produced by eugenol was significantly smaller (p<0.05) than carvacrol and thymol (Mith *et al.,* 2014) [17].

Minimum Inhibitory Concentration (MIC) of selected essential oils

The results for Minimum Inhibitory Concentration (MIC) of essential oils against the foodborne microbes by disc diffusion and macro-tube dilution method are presented in Table 2. In general, the MIC of oregano essential oil was significantly lower (p<0.05) than clove essential oil against selected microorganisms. For oregano essential oil, *E. coli* was the most sensitive, while *Salmonella* Typhimurium and *Staphylococcus aureus* were equally susceptible. *Pseudomonas aeruginosa* was the most resistant against oregano essential oil, while clove essential oil was found ineffectual against it. Clove essential oil was the most effective to *Salmonella* Typhimurium, while it was equally susceptible to *E. coli* and *Staphylococcus aureus*. The resistance of *Pseudomonas aeruginosa* towards several antibiotics and essential oils could be attributed to its pretty impermeable membrane, constitutively expressed and inducible efflux systems, and a chromosomally encoded inducible β-lactamase system (El-Hosseiny *et al.,* 2014) [32].

Table 2: MICs of essential oils tested *in-vitro* against foodborne microorganisms using disc diffusion method (µl/disc) and macro-tube dilution method (µl/ml of BHI broth)

	Disc diffusion method EOs (µl/disc)		Macro-tube dilution method (µl/ml of BHI broth)	
Microorganisms	**Clove EO**	**Oregano EO**	**Clove EO**	**Oregano EO**
Salmonella Typhimurium (MTCC 98)	0.125^{b}	0.03125^{a}	0.5^{b}	0.05^{a}
Staphylococcus aureus (MTCC 902)	0.25^{b}	0.03125^{a}	0.4^{b}	0.1^{a}
Pseudomonas aeruginosa (MTCC 2453)	No inhibition even in 10µl	1.0	Negative	2.0
E. coli (MTCC 723)	0.25^{b}	0.015625^{a}	0.5^{b}	0.05^{a}

n=6; Mean with different superscripts differ significantly (p<0.05)

The macro-tube dilution also reported a significantly higher (p<0.05) efficacy of oregano essential oil than clove essential oil. Oregano essential oil was equally inhibitory to *Salmonella* Typhimurium and *E. coli*, followed by *Staphylococcus aureus*, while *Pseudomonas aeruginosa* was the most resistant. *Salmonella* Typhimurium and *E. coli* were equally susceptible to clove EO, while *Staphylococcus aureus* was more susceptible than both of them. Similarly, oregano reported the highest antimicrobial activity among different essential oils against various foodborne pathogens (Sokovic *et al.,* 2010; Hussain *et al*., 2008) [19, 29].

The results obtained in the present study could be attributed to the inhibitory action of essential oils, which is related to hydrophobicity. The hydrophobicity is directly associated with the partitioning behaviour of the lipophilic compounds in octanol/water (log P) and their partition in the cytoplasmic microbial membranes (Lanciotti *et al.,* 2003) [33]. The compound with the most hydrophobic properties tends to be the most toxic, with the cytoplasmic membrane as the primary site of toxicity (Sikkema *et al.,* 1995; Weber and de Bont 1996) [34, 35]. The lipophilic compounds possess a high affinity for cytoplasmic membranes, and their insertions induce lipid ordering and bilayer stability, resulting in a decrease in the membrane integrity and anincrease of passive proton flux across the membrane. This effect is notably reported with the compound having logP higher than 3. In this context, carvacrol, with a logP of 3.52, was found to be the most efficient antimicrobial compound. In contrast, eugenol with logP of less than 3 showed a lower efficiency than carvacrol towards *E. coli*, *Staph. aureus*, *B. subtilis* and *S. cerevisiae* (Ben Arfa *et al.*, 2006) [36].

Moreover, the weak antimicrobial effect of eugenol could also be due to the presence of a methoxyl group in the ortho position, inhibiting the OH group from releasing its proton quickly and limiting proton exchanging ability which in turn reduces the antimicrobial activity (Ben Arfa *et al*., 2006).) [36]. It has been reported that compounds with a logP higher than 4 are generally non toxic, as they cannot reach a toxic concentration in the cell membrane due to their insolubility. Carvacrol, on the other hand, due to its appropriate hydrophobicity, presence of delocalized electron system and hydroxyl group could be accumulated in the cell membrane and its proton-release ability may induce conformational modification of the membrane, depletion of ATP pool and eventually tothe cell death (Vermue *et al.,* 1993) [37].

Pseudomonas aeruginosa MTCC 2453 was the most resistant of all the food bornemicrobes challenged in the present study. It was found that *Pseudomonas aeruginosa* and *Proteus mirabilis* were the most resistant among several tested species. In contrast, high antibacterial activity was found with oregano essential oil and its principal component, carvacrol, against *Pseudomonas spp.* (Sokovic *et al.,* 2010) [19]. It has also been reported that the MIC of *Origanum compactum* against *Pseudomonas fluorescens* was 1 µl/ml, which was twice that of other foodborne microbes (Mith *et al.*, 2014**)** [17]. Similar results were also observed where thyme essential oil containing thymol had higher antipseudomonal activity when tested against different conventional antibiotics (Clarke,

2008) [31]. The higher antimicrobial activity of some of the essential oil could be attributed to the presence of oxygenated terpene components (El-Hosseiny *et al.,* 2014) [32]. These findings corroborated with the results obtained in the present study where oregano essential oil containing carvacrol, an oxygenated terpene, had more antimicrobial activity than clove essential oil containing eugenol, a non-terpenoid compound (Clarke, 2008) [31].

Minimum Bactericidal Concentration (MBC) of selected EOs

The results related to MBC of clove and oregano essential oils against the selectedfood borne microorganisms are presented in figure 2. The results revealed that oregano was significantly inhibitory (p<0.05) to selected microorganisms than clove essential oil. Among the selected food borne microorganisms, *Pseudomonas aeruginosa* was the most resistant to both essential oils.

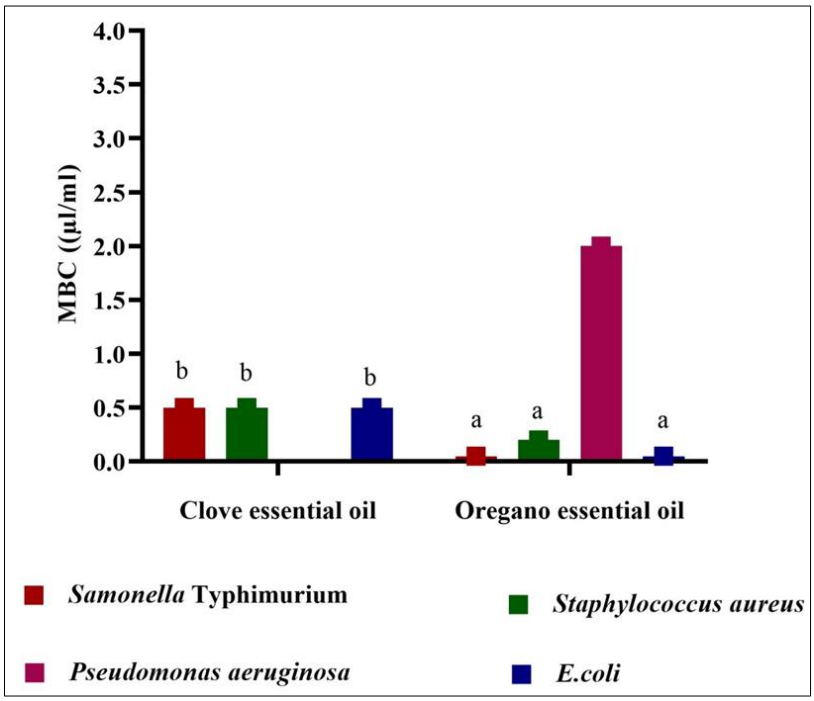

Fig 2: MBCs of essential oils tested *in-vitro* against foodborne microorganisms. n=6; Mean with different superscripts differ significantly (p<0.05).

Clove essential oil was ineffective against the *Pseudomonas aeruginosa*, while oregano essentialoil had an MBC of 2µl/ml, which was significantly higher p<0.05) than other selected microorganisms. The increased resistance of *Pseudomonas aeruginosa* could be attributed to the presence of an active efflux mechanism and the barrier function located in the outer membrane lipopolysaccharide, which screens out and restricts entry of antimicrobial agents or substances inside the cell (Cox and Markham, 2007) [38].

Conclusions

The antimicrobial activity of oregano essential oil was comparable with the selected antibiotics**.** However, it exhibited significantly higher (p<0.05) antimicrobial activity than selected antibiotics against *E. coli*. In contrast, its antimicrobial activity was comparable to fourth generation antibiotics against *Staphylococcus aureus* and *Salmonella* Typhimurium. However, clove essential exhibited significantly lower (p<0.05) antimicrobial activity than selected antibiotics. The MIC revealed that antimicrobial activity of oregano essential oil was significantly higher (p<0.05) than clove essential oil against selected foodborne microorgaisms. Among the selected microorganisms, *Pseudomonas aeruginosa* was the most resistant, as revealed by the MIC and MBC. Thus, from the above results, it could be concluded that oregano essential oil has potent antimicrobial activity comparable to that of the selected antibiotics. It can be successfully utilized as natural preservatives to preserve meat and meat products without any detrimental effects on consumers health and improvement of microbial quality and shelf-life of the products.

Disclosure Statement

There is no conflict of interest among the authors.

References

1. Marques CS, Carvalho SG, Bertoli LD, Villanova JCO, Pinheiro PF, dos Santos DCM, *et al*. Bernardes, β-Cyclodextrin inclusion complexes with essential oils: Obtention, characterization, antimicrobial activity and potential application for food preservative sachets. Food Res. Int. 2019;119:499-509 DOI: https://doi.org/10.1016/j.foodres.2019.01.016.
2. Maria NG-C Jp P-R, Heber G-M. Sauces, spices, and condiments: definitions, potential benefits, consumption patterns, and global markets. Annals of the New York Academy of Sciences. 2016;1379:3-16. DOI: 10.1111/nyas.13045
3. Bhavaniramya S, Vishnupriya S, Al-Aboody MS, Vijayakumar R, Baskaran D. Role of essential oils in food safety: Antimicrobial and antioxidant applications. Grain & Oil Science and Technology. 2019;2(2):49-55. DOI: https://doi.org/10.1016/j.gaost.2019.03.001
4. Radhakrishnan K, Babuskin S, Azhagu Saravana Babu P, Sasikala M, Sabina K, Archana G, *et al*. Antimicrobial and antioxidant effects of spice extracts on the shelf life

extension of raw chicken meat. International Journal of Food Microbiology. 2014;171:32-40.
5. Muppalla SR, Kanatt SR, Chawla SP, Sharma A. Carboxymethyl cellulose–polyvinylalcohol films with clove oil for active packaging of ground chicken meat. Food Packaging and Shelf Life. 2014;2:51-58.
6. Petrou S, Tsiraki M, Giatrakou V, Savvaidis IN. Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. International Journal of Food Microbiology. 2012;156(3):264-271.
7. Sojic B, Pavli CB, Ikoni´ CP, Tomovi´c V, Ikoni´c B, Zekovi´c Z, *et al*. Coriander essential oil as natural food additive improves the quality and safety of cooked pork sausages with different nitrite levels. Meat Science. 2019;157:107879. https://doi.org/10.1016/j.meatsci.2019.107879.
8. Pabast M, Shariatifar N, Beikzadeh S, Jahed G. Effects of chitosan coatings incorporating with free or nano-encapsulated Satureja plant essential oil on quality characteristics of lamb meat. Food Control. 2018;91:185-192.
9. Pesavento G, Calonico C, Bilia AR, Barnabei M, Calesini FR, Addona L, *et al*. Antibacterial activity of Oregano, Rosmarinus and Thymus essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meat balls. Food Control. 2015;54:188-199.
10. Radunz M, da Trindade MLM, Camargo TM, Radünz AL, Borges CD, Gandra EA, *et al*. Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (*Syzygium aromaticum* L.) essential oil. Food Chemistry. 2019;276:180-186. https://doi.org/10.1016/j.foodchem.2018.09.173.
11. Nikmaram N, Budaraju S, Barba FJ, Lorenzo JM, Cox RB, Mallikarjunan K, *et al*. Application of plant extracts to improve the shelf-life, nutritional and health-related properties of ready-to-eat meat products. Meat Science, 2018, 245-255p.
12. Patel S, Gogna P, Tapping botanicals for essential oils: Progress and hurdles in cancer mitigation. Industrial Crops Products. 2015;76:1148-1163.
13. Zuzarte M, Salgueiro L. Essential oils chemistry, In Bioactive Essential Oils and Cancer; De Sousa, D.P., Ed.; Springer International Publishing: Cham, Switzerland, 2015, 19-61.
14. Islam MT, Da Mata AMOF, de Aguiar RPS, Paz MFCJ, de Alencar MVOB, Ferreira PMP, *et al*. Melo-Cavalcante, Therapeutic potential of essential oils focusing on diterpenes. Phytotherapy Research. 2016;30:1420-1444.
15. Lenard*a*o EJ, Savegnago L, Jacob RG, Victoria FN, Martinez DM. Antinociceptive effect of essential oils and their constituents: An update review. Journal of Brazilian Chemical Society. 2016;27:435-474.
16. Dima C, Dima S. Essential oils in foods: Extraction, stabilization, and toxicity, Current Opinion in Food Science. 2015;5:29-35.
17. Mith H, Remi D, Veronique D, Abdesselam Z, Georges D, Antoine C. Antimicrobial activities of commercial essential oils and their components against foodborne pathogens and food spoilage bacteria. Food Science and Nutrition. 2014;2(4):403-416.
18. Pinheiro PF, Costa AV, Alves TA, Galter IN, Pinheiro CA, Pereira AF, *et al*. Phytotoxicity and cytotoxicity of essential oil from leaves of *Plectranthus amboinicus*, carvacrol, and thymol in plant bioassays, Journal of Agricultural and Food Chemistry. 2015;63:8981e8990.
19. Sokovic M, Glamoclija Marin JPD, Brkic D, Van Griensven LJLD. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in-vitro model, Molecules. 2010;15:7532-7546.
20. Bassanetti I, Carcelli M, Buschini A, Montalbano S, Leonardi G, Pelagatti P, *et al*. Investigation of antibacterial activity of new classes of essential oils derivatives, Food Control, Food Control xxx, 2016, 1e7.
21. Sarac N, Ugur A. Antimicrobial activities of the essential oils of *Origanum onites* L., *Origanum vulgare* L subspecies hirtum (Link) Ietswaart, *Satureja thymbra* L., and *Thymus cilicicus* Boiss. & Bal. growing wild in Turkey, Journal of Medicinal Food. 2008;11:568-573.
22. Mechergui KJA, Coelho MC, Serra SB, Lamine S, Boukhchina, Khouja ML. Essential oils of *Origanum vulgare* L. subsp. *glandulosum* (Desf.) Ietswaart from Tunisia: chemical composition and antioxidant activity. Journal of Science of Food and Agriculture. 2010;90:1745-1749.
23. Di Cesare LF, Forni E, Viscardi D, Nani RC. Changes in the chemical composition of basil caused by different drying procedures. Journal of Agricultural and Food Chemistry. 2003;51:3575-3581.
24. Burt S. Essential oils: their antibacterial properties and potential applications in foods: A review, International Journal of Food Microbiology. 2004;94:223-253.
25. Karakaya S, El SN, Karagozl N, Sahin S. Antioxidant and antimicrobial activities of essential oils obtained from oregano (*Origanum vulgare* spp. *hirtum*) by using different extraction methods, Journal of Medicinal Food. 2011;14:645-652.
26. Sharma H, Mendiratta SK, Agrawal RK, Gurunathan K, Kumarand Singh S, TP. Use of various essential oils as bio preservatives and their effect on the quality of vacuum-packaged fresh chicken sausages under frozen conditions. LWT-Food Science and Technology. 2017;81:118-127.
27. Steel RGD, Torris JH. Principles and Procedures of Statistics: A biometrical approach. 2nded. Mcgraw Hill International Book Co. 1981, 173-175.
28. Donaldson JR, Steven L, Warner Cates RG, Gary Young D. Assessment of Antimicrobial Activity of Fourteen Essential Oils When Using Dilution and Diffusion Methods. Pharmaceutical Biology. 2005;43(8):687-695.
29. Hussain AIF, Anwar STH, Sherazi, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chemistry. 2008;108:986-995.
30. Castilho PC, Savluchinske-Feio S, Weinhold TS, Gouveia SC. Evaluation of the antimicrobial and antioxidant activities of essential oils, extracts and their main components from oregano from Madeira Island, Portugal. Food Control. 2012;23:552-558.
31. Clarke S. Essential Chemistry for Aromotherapy. Churchill Livingstone, 2008. https://doi.org/10.1016/B978-0-443-10403-9.X0001-5
32. El-Hosseiny L, El-Shenawy M, Haroun M, Abdullah F. Comparative Evaluation of the Inhibitory Effect of Some Essential Oils with Antibiotics against Pseudomonas aeruginosa. International Journal of Antibiotics, 2014.

http://dx.doi.org/10.1155/2014/586252.
33. Lanciotti R, Belleti N, Patrignani F, Gianotti A, Gardini F, Guerzoni ME. Application of hexanal, (E)-2-hexanal, and hexyl Acetate to improve the safety of fresh –sliced-apples. Journal of Agriculture and Food Chemistry. 2003;51:2958-2963.
34. Sikkema J, De Bont J, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiological Reviews. 1995;59:201-222.
35. Weber FJ, Jam de Bont. Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. Biochimica et Biophysica Acta. 1996;1286:225-245.
36. Ben Arfa A, Combes S, Preziosi-Belloy L, Gontard N, Chalier P. Antimicrobial activity of carvacrol related to its chemical structure. Letters in Applied Microbiology. 2006;43:149-154.
37. Vermue M, Sikkema J, Verheul A, Bakker R, Tramper J. Toxicity of homologous series of organic-solvents for the Gram-positive bacteria *Arthrobacter* and *Nocardia* sp and the Gram-negative bacteria Acinetobacter and Pseudomonas sp. Biotechnology and Bioengineering. 1993;42:747-758.
38. Cox SD, Markham JL. Susceptibility and intrinsic tolerance of Pseudomonas aeruginosa to selected plant volatile compounds. Journal of Applied Microbiology. 2007;103(4):930-936.
DOI: 10.1111/j.1365-2672.2007.03353.x