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Research article

Essential oils for controlling multidrug-resistant pathogens in livestock: bridging *in-vitro* data to phytogenic additives

Madhu Mishra[1], Deepak Upadhyay[2*], Ravi Kant Agrawal[3], G. Bhuvana Priya[4] and Anubha Pathak[5]

[1]Veterinary Assistant Surgeon, Govt. of Madhya Pradesh, Niwari- 472442, India
[2]College of Veterinary and Animal Sciences, RLBCAU, Nauner- 475661, India
[3]ICAR-Indian Veterinary Research Institute, Izzatnagar-243122, India
[4]College of Agriculture, Kyrdemkulai, CAU, Imphal-793105, India
[5]ICAR-National Institute of High Security Animal Diseases, Bhopal-462022, India
[*]Corresponding author email: dpkvet@gmail.com

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Abstract

Antibiotic resistance is a pressing global crisis fueled by overuse in medicine, farming, and agriculture, producing superbugs like MRSA (Methicillin-resistant Staphylococcus aureus) and extended-spectrum-lactamase (ESBL) *E. coli*, causing prolonged and untreatable infections. Essential oils (EO) provide a natural alternative, offering broad-spectrum antibacterial potency with reduced risk of rapid resistance. Present study assessed the antimicrobial efficacy of 71 essential oils against 144 clinical bacterial isolates by determining their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as compared with 14 standard antibiotics. Twelve EOs such as thyme, holy basil, lemon grass, cinnamon, oregano, rosewood, cassia, rose, clove, palmrosa, carom seeds and betel leaf showed remarkable potency. Out of 12 Eos, thyme, cinnamon, cassia, oregano and carom seeds were found to be most effective and categorized as strong while holy basil, lemongrass, rosewood, palmrosa, rose, clove and betel leaf were considered as moderate effective EOs. Notably, oregano achieved the lowest MIC (0.039 μL) against *E. coli* and the lowest MBC (0.156 μL) against *Klebsiella pneumoniae*. EOs demonstrates powerful antibacterial effects against multidrug-resistant pathogens, frequently surpassing conventional antibiotics in efficacy. Judicious use of these natural oils as feed additives can effectively contribute to the livestock health.

Keywords: Antimicrobial resistance, Bioactive compounds, Essential oils, ESBL, MRSA, Minimum inhibitory concentration, Natural antimicrobials.

Introduction

Antibiotic resistance has emerged as one of the most critical global health emergencies, significantly impacting the sustainability of livestock production systems. The indiscriminate use of antibiotics as growth promoters and prophylactic agents in veterinary practice and livestock farming has created powerful selective pressure, driving bacteria to evolve sophisticated resistance mechanisms at an alarming rate (CDC, 2013; WHO, 2014). This "One Health" crisis not only threatens recovery from infections but also compromises the economic viability of rangeland-based animal husbandry. Multidrug-resistant pathogens, *viz.* methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, carbapenem-resistant Enterobacteriaceae, vancomycin-resistant enterococci, *etc*., are now widespread in agricultural environments. In livestock, these resistant strains lead to expensive treatments, prolonged recovery periods, and significantly higher mortality rates from common infections like pneumonia (WHO, 2014). As the World Health Organization warns of a "post-antibiotic era," the livestock sector faces an urgent mandate to transition toward "green" antimicrobial strategies that safeguard both animal health and environmental integrity.

In the search for sustainable alternatives, the biodiversity of rangelands and agroforestry systems offers a promising frontier. Essential oils (EOs), volatile secondary metabolites synthesized by aromatic plants, are emerging as effective natural feed additives. These plant-derived compounds provide a holistic alternative to synthetic growth promoters, aligning with the goals of organic and low-input livestock systems (Jiang *et al.*, 2024). Many EOs display potent broad-spectrum antibacterial, antifungal, and antibiofilm activity (Bassolé and Juliani, 2012). The antimicrobial efficacy of EOs is attributed to complex mixtures of phenolic compounds (thymol, carvacrol), aldehydes, and terpenes. Their bioactive compounds exert potent antimicrobial and antiparasitic effects by disrupting microbial cell membranes, causing leakage of cellular contents, and selectively suppressing pathogens such as *E. coli*, *Salmonella*, *Clostridium*, and protozoa while preserving beneficial gut bacteria like *Lactobacillus* (Abdelli *et al.*, 2021). This reduces enteric disease risk and parasite burdens in poultry and ruminants. Studies confirm efficacy against multidrug-resistant strains, including MRSA, ESBL-producers, and foodborne pathogens (Bassolé and Juliani, 2012; Yap *et al*., 2014). Beyond direct antimicrobial action, EOs derived from plants deliver potent antioxidant and anti-inflammatory benefits, enhancing nutrient absorption and improving meat and milk quality (Nehme *et al*., 2021; Putra *et al.*, 2024). For ruminants, EOs optimize rumen fermentation by inhibiting methanogens and hyper-ammonia producers, shifting volatile fatty acid profiles toward propionate, and cutting methane emissions by 10–50% (Benchaar and Hassanat, 2025). Overall, these mechanisms translate to improved growth performance, average daily gain, feed efficiency and digestibility, often matching or exceeding antibiotic effects, supporting healthier, more efficient, and environmentally friendly livestock systems (Putra *et al*., 2024). Unlike single-target synthetic antibiotics, the complex chemical profile of EOs disrupts multiple cellular processes, including efflux pumps and quorum sensing, thereby slowing the rate of resistance development (Yap *et al.*, 2014). Challenges like standardization, volatility, organoleptic effects, and formulation persist, yet EOs offer an eco-friendly route to curb antibiotic reliance and combat antimicrobial resistance.

Despite their potential, the application of these rangeland-derived metabolites requires rigorous validation against contemporary MDR strains found in livestock settings. Therefore, the present study evaluated the antimicrobial efficacy of selected Essential Oils compared to conventional antibiotics against multidrug-resistant bacterial species, aiming to provide a scientific basis for their integration into sustainable agroforestry and range management practices.

Materials and Methods

Bacterial strains: Bacterial isolates used were obtained from the Food Microbiology Laboratory (Division of Livestock Products Technology) and other sections of ICAR-Indian Veterinary Research Institute (IVRI), Izzatnagar. A total of 144 isolates, comprising 15 strains each of the foodborne pathogens *Klebsiella pneumoniae*, *Vibrio parahaemolyticus*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Salmonella pullorum*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Proteus* spp.; 6 reference strains of *Staphylococcus aureus* (MTCC 96, ATCC 700699, ATCC 25923, ATCC 43300, ATCC 29213, and FRI 722); 9 Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates; 62 Extended-Spectrum β-Lactamase (ESBL)-producing *Escherichia coli* isolates; and 58 field isolates of *Staphylococcus* spp. were studied.

For revival, isolates were inoculated into brain heart infusion (BHI) broth and incubated overnight at 37°C. Subsequently, they were streaked onto appropriate selective media (e.g., mannitol salt agar for *Staphylococcus* spp., MacConkey agar for *E. coli*, TCBS agar for *V. parahaemolyticus*, and others as applicable). Isolates were identified through Gram staining and direct microscopic examination, standard biochemical tests, and species-specific PCR assays.

Preparation of discs containing different EO: For antimicrobial susceptibility screening, sterile 6 mm filter paper discs were impregnated with 5 μL of each EO dissolved in methanol. The discs were then placed on the agar surface after complete absorption and solvent evaporation. 71 EOs used in the study are listed in Table 1.

Screening for antimicrobial activity: All revived bacterial isolates were screened for susceptibility to 71 EOs using the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966), a standardized direct-contact technique on solid agar medium for assessing antibacterial activity. Mueller-Hinton agar (MHA) served as the primary medium for the antimicrobial susceptibility testing. However, for bacteria that exhibit poor or no growth on MHA (e.g., *Vibrio parahaemolyticus*), alternative suitable media were employed. Overnight cultures of each bacterial isolate were prepared by inoculating a single colony into 10 mL of brain heart infusion (BHI) broth, followed by incubation at 37°C with shaking (200 rpm) in a shaker incubator. A 200 μL aliquot of the adjusted bacterial suspension was then spread evenly onto MHA (or blood agar/appropriate medium) plates using a sterile L-shaped spreader. Plates were allowed to dry for 10 minutes at room temperature. EOs impregnated discs were subsequently placed equidistantly on the agar surface using sterile forceps. Following incubation at 37°C for 24 hours, the zones of inhibition (ZOI) were measured using a calibrated scale, including the 6 mm diameter of the disc itself. The antimicrobial activity of the tested substances was classified according to the diameter of the zone of inhibition (ZOI) as: strong activity (ZOI >20), moderate activity (ZOI 15-20), weak activity (ZOI 10-15), and inefficient/negative (ZOI ≤10).

Table 1. List of 71 essential oils screened for antimicrobial activity

S. No.	Essential oil	Botanical name	S. No.	Essential oil	Botanical name	S. No.	Essential oil	Botanical name
1	Niaouli oil	*Melaleuca quinquenervia*	**25**	Juniper Berry oil	*Juniperus*	**49**	Cumin oil	*Cuminum cyminum*
2	Tea-Tree oil	*Melaleuca alternifolia*	**26**	Geranium oil	*Pelargonium hortorum*	**50**	Cajeput oil	*Melaleuca cajuputi*
3	Bergamot oil	*Citrus bergamia*	**27**	Anise oil	*Pimpinella anisum*	**51**	Laurel Leaf oil	*Laurus nobilis*
4	Grapefruit oil	*Citrus paradisi*	**28**	Rosemary oil	*Rosmarinus officinalis*	**52**	Lavender oil	*Lavandula*
5	Pine oil	*Pinus*	**29**	Neem oil	*Azadirachta indica*	**53**	Mandarin oil	*Citrus reticulata*
6	Bay oil	*Laurus nobilis*	**30**	Calamus oil	*Acorus calamus*	**54**	Tagetes oil	*Tagetes erecta*
7	Sage oil	*Salvia officinalis*	**31**	Spearmint oil	*Mentha spicata*	**55**	Pine oil	*Pinus roxburghii*
8	Thyme oil	*Thymus vulgaris*	**32**	Cedarwood oil	*Cedrus deodara*	**56**	Myrtle oil	*Lagerstroemia speciosa*
9	Citronella oil	*Cymbopogon*	**33**	Ginger oil	*Zingiber officinale*	**57**	Frankincense oil	*Boswellia serrata*
10	Holy Basil oil	*Ocimum tenuiflorum; / Ocimum sanctum*	**34**	Orange oil	*Citrus sinensis*	**58**	Cade oil	*Juniperus oxycedrus*
11	Lemongrass oil	*Cymbopogon flexuosus*	**35**	Peppermint oil	*Mentha piperita*	**59**	Tuberose oil	*Polianthes tuberosa*
12	Cinnamon oil	*Cinnamomum verum*	**36**	Cassia oil	*Cassia fistula*	**60**	Carom seeds oil	*Trachyspermumammi*
13	Lemon oil	*Citrus limon*	**37**	Rose oil	*Hibiscus syriacus*	**61**	Carrot seed oil	*Daucus carota*
14	Lime oil	*Citrus aurantifolia*	**38**	Calendula oil	*Calendula officinalis*	**62**	Camphor oil	*Cinnamomum camphora*
15	Caraway oil	*Carum carvi*	**39**	Perilla oil	*Perilla frutescens*	**63**	Neroli oil	*Citrus aurantium*
16	Nutmeg oil	*Myristica fragrans*	**40**	Black Pepper oil	*Piper nigrum*	**64**	Ylang Ylang oil	*Cananga odorata*
17	Eucalyptus oil	*Eucalyptus globulus*	**41**	Clove oil	*Syzygium aromaticum*	**65**	Petitgrain oil	*Citrus aurantium*
18	Patchouli oil	*Pogostemon cablin*	**42**	Turmeric oil	*Curcuma longa*	**66**	Betel leaf oil	*Piper betle*
19	Oregano oil	*Origanum vulgare*	**43**	Chamomile oil	*Matricaria chamomilla*	**67**	Vetiver oil	*Chrysopogon zizanioides*
20	Rosewood oil	*Dalbergia sissoo*	**44**	Dill oil	*Anethum graveolens*	**68**	Mace oil	*Myristica fragrans*
21	Melissa oil	*Melissa officinalis*	**45**	Wintergreen oil	*Gaultheria procumbens*	**69**	Cyperiol oil	*Cyperus scariosus*
22	Coriander oil	*Coriandrum sativum*	**46**	Aloe Vera oil	*Aloe barbadensis*	**70**	Curry leaf oil	*Murraya koenigii*
23	Marjoram oil	*Origanum majorana*	**47**	Palmarosa oil	*Cymbopogon martinii*	**71**	Galbanum oil	*Ferula galbaniflua*
24	Garlic oil	*Allium sativum*	**48**	Fennel oil	*Foeniculum vulgare*			

***Determination of minimum inhibitory concentration (MIC)**:* For MIC estimation of thyme, holy basil, lemongrass, cinnamon, oregano, rosewood, cassia, rose, clove, palmarosa, carom seeds, and betel leaf, the disc dilution method was used. Discs (6 mm) were impregnated with serially diluted EOs in methanol at volumes of 5, 2.5, 1.25, 0.625, 0.3125, 0.156, and 0.078 µL. Standardized overnight cultures (~10^5–10^6 CFU/mL) were spread (200 µL) onto Mueller-Hinton agar (MHA) or blood agar (BA) plates, followed by placement of the discs, incubation at 37°C for 24 hours, and measurement of inhibition zones. The MIC was defined as the lowest volume producing a detectable zone of inhibition.

***Determination of minimum bactericidal concentration (MBC)**:* MBC of selected broad-spectrum EOs (thyme, holy basil, cinnamon, oregano, cassia, clove, carom seeds, and betel leaf) was determined by broth microdilution followed by subculture. EOs were serially diluted (1:2 to 1:1024) in DMSO across wells 1–10 of a microtitre plate. About 20 µL of each dilution was added to 180 µL of standardized overnight culture and incubated at 37°C for 24 hours. Subsequently, 100 µL from wells 5 to 10 was plated onto MHA or BA and incubated for 24 h at 37°C. The MBC was recorded as the lowest EO concentration, showing no colony growth on the subculture plates.

***Determination of antimicrobial resistance pattern of microbes towards conventional antibiotics*:** Antibiotic susceptibility testing was performed using 14 broad-spectrum and next-generation antibiotics: ampicillin (10 mcg/disc), amoxyclav (30 mcg/disc), azithromycin (15 mcg/disc), cefoxitin (30 mcg/disc), cloxacillin (1 mcg/disc), erythromycin (15 mcg/disc), fosfomycin (200 mcg/disc), gentamycin (10 mcg/disc), meropenem (10 mcg/disc), minocycline (30 mcg/disc), clindamycin (2 mcg/disc) teicoplanin (30 mcg/disc), tigecycline (15 mcg/disc) and vancomycin (30 mcg/disc) All discs were obtained from HiMedia, India.

Results and Discussion

Antibiotic resistance profile against antibiotic discs (as per Clinical and Laboratory Standards Institute (CLSI)

Table 2. Antibiotic resistance profile against antibiotic discs among foodborne pathogens

Bacterial Strain	**Antibiotic**	**Resistance percentage / Status**	**Resistance criterion (Zone of Inhibition)**
Staphylococcus aureus	Ampicillin	83.3% (5/6)	ZOI< 28 mm
S. aureus	Amoxyclav	33.3% (2/6)	ZOI < 19 mm
S. aureus	Cefoxitin	33.3% (2/6)	ZOI < 21 mm
S. aureus	Clindamycin	33.3% (2/6)	ZOI < 14 mm
S. aureus	Erythromycin	50% (3/6)	ZOI < 13 mm
S. aureus	Gentamicin	16.6% (1/6)	ZOI < 12 mm
S. aureus	Minocycline	16.6% (1/6)	ZOI < 14 mm
Llisteria monocytogenes	Erythromycin	Resistant	ZOI < 25 mm
Escherichia coli O157:H7	Fosfomycin	Resistant	ZOI < 12 mm
E. coli O157:H7	Gentamicin	Resistant	ZOI < 12 mm
E. coli O157:H7	Minocycline	Resistant	ZOI < 12 mm
E. coli O157:H7	Tigecycline	Resistant	ZOI < 15 mm
E. coli O157:H7	Meropenem	Resistant	ZOI < 16 mm
Bacillus cereus	Cefoxitin	No ZOI (resistant)	No inhibition
B. cereus	Clindamycin	No ZOI (resistant)	No inhibition
B. cereus	Cloxacillin	No ZOI (resistant)	No inhibition
B. cereus	Erythromycin	No ZOI (resistant)	No inhibition
B. cereus	Teicoplanin	No ZOI (resistant)	No inhibition
B. cereus	Vancomycin	No ZOI (resistant)	No inhibition
Vibrio parahaemolyticus	Cloxacillin	No ZOI (resistant)	No inhibition
V. parahaemolyticus	Teicoplanin	No ZOI (resistant)	No inhibition
V. parahaemolyticus	Vancomycin	No ZOI (resistant)	No inhibition

Table 3. Antibacterial activity of EOs against foodborne pathogens

Bacterial strain	Essential oils showing activity
B. cereus	Thyme, Cinnamon, Oregano, Cassia
V. parahaemolyticus	Thyme, Cinnamon, Oregano, Cassia, Palmarosa
Salmonella typhimurium	Cinnamon, Cassia
Salmonella pullorum	Thyme, Holy basil, Lemongrass, Cinnamon, Cassia
L. monocytogenes	Thyme, Cinnamon
E. coli O157:H7	Cinnamon, Cassia
Klebsiella. pneumoniae	Thyme, Cinnamon, Cassia
Yersinia enterocolitica	Thyme, Holy basil, Cinnamon, Oregano, Rosewood, Carom seeds

and European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards) among foodborne pathogens is given in Table 2.

EOs with strong activity (ZOI> 20 mm) against foodborne pathogens: Activity of EOs against foodborne pathogens is shown in Table 3.

Food safety continues to be a pressing global issue, as microbial contamination in perishable foods like meat causes foodborne illnesses and spoilage, manifesting as off-odors, discoloration, gas production, and slime formation. Plant-derived EOs, abundant in bioactive compounds such as thymol, carvacrol, and cinnamaldehyde, provide a compelling natural alternative due to their broad-spectrum antimicrobial activity, chemical diversity, and long-standing traditional use (Yap *et al*., 2014; Stefanakis *et al*., 2013). EOs producing a zone of inhibition (ZOI) >20 mm were selected for direct comparison with antibiotics. Fig. 1 illustrates the sensitivity patterns of antibiotics versus EOs against food-poisoning-associated *Staphylococcus* spp. Phenolic-rich EOs (thyme, oregano, cinnamon, cassia, carom seeds) demonstrated superior efficacy due to potent membrane-disrupting mechanisms, outperforming less active oils that showed more selective or strain-specific effects. Particularly, thyme, cinnamon, oregano, and cassia EOs exhibited strong, consistent inhibition against resistant strains, including *S. aureus* showing high resistance to ampicillin (83.3%) and moderate resistance to cefoxitin, clindamycin, and erythromycin (33.3%).

Antibiotic resistance profile among the MRSA isolates (n=9) as per (CLSI and EUCAST standards)***:*** Antibiotic resistance profile against antibiotic discs) among MRSA is given in table 4.

EOs with strong activity (ZOI > 20 mm) against MRSA isolates: Activity of EOs against MRSA is shown in Table 5.

Comparative sensitivity patterns of antibiotics versus EOs against MRSA isolates are presented as bar diagrams in Fig 2.

Antibiotic susceptibility testing on 9 clinical MRSA isolates uncovered significant multidrug resistance, with 88.8% exhibiting resistance to ampicillin, cefoxitin, and clindamycin based on CLSI/EUCAST standards. Lower levels of resistance were observed to amoxiclav, gentamicin, minocycline, azithromycin, and tigecycline. These patterns emphasize the increasing challenges in managing MRSA infections within healthcare settings, where conventional antibiotics often prove inadequate.

Conversely, EOs showed notable promise against these resistant strains. Oregano demonstrated strong antimicrobial effects, with zones of inhibition exceeding 20 mm against 66.6% of the isolates. Fig. 2 illustrates the sensitivity patterns of antibiotics versus EOs against MRSA. Cinnamon and cassia each impacted 44.4%, while thyme affected 33.3%. Such outcomes suggest that oregano, cinnamon, and cassia could serve as valuable natural alternatives to traditional antibiotics in addressing multidrug-resistant MRSA (Xiao *et al*., 2020).

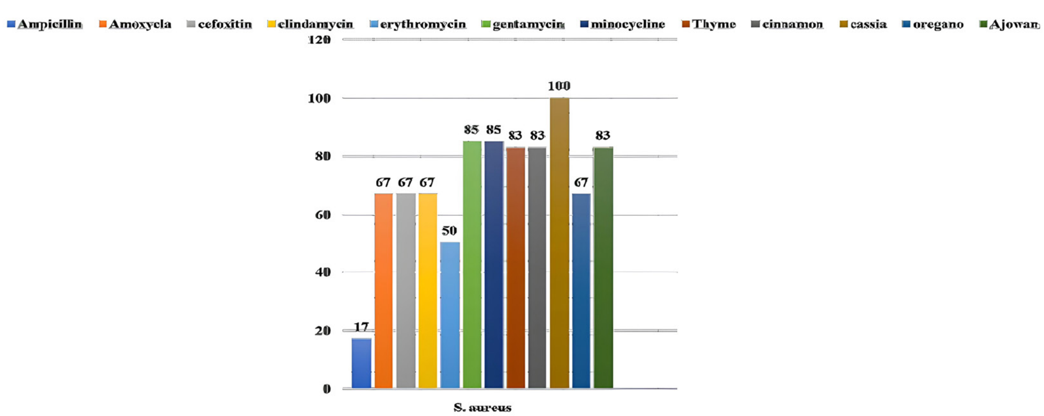

Fig 1. Comparison of effectivity of antibiotics and most effective EOs against *S. aureus*

Table 4. Antibiotic resistance profile against antibiotic discs among MRSA

Bacterial Strains	Antibiotic	Percentage resistant	Resistance criteria (ZOI)
MRSA	Ampicillin	88.8% (8/9)	ZOI< 28 mm
MRSA	Cefoxitin	88.8%(8/9)	ZOI< 21 mm
MRSA	Clindamycin	88.8%(8/9)	ZOI< 14 mm
MRSA	Amoxyclav	22.2%(2/9)	ZOI< 19 mm
MRSA	Gentamicin	22.2%(2/9)	ZOI< 12 mm
MRSA	Minocycline	11.1%(1/9)	ZOI< 14 mm
MRSA	Azithromycin	11.1%(1/9)	ZOI< 13 mm
MRSA	Tigecycline	11.1%(1/9)	ZOI< 18 mm

Antibiotic resistance profile among the ESBL E. coli* isolates (n=62*): Antibiotic resistance profile against antibiotic discs (as per CLSI and EUCAST standards) among ESBL *E. coli* isolates is shown in Table 6.

EOs with strong activity (ZOI > 20 mm) against ESBL E. coli isolates: Activity of EOs against *ESBL E. coli* is shown in Table 7

Comparative sensitivity patterns of antibiotics versus EOs against ESBL *E. coli* isolates are presented as bar diagrams in Fig 3.

Antibiotic susceptibility testing of 62 resistant gram-positive isolates revealed high resistance to ampicillin (88.7%), followed by amoxiclav (41.93%), minocycline (38.7%), and meropenem (29.03%), confirming the critical threat of multidrug resistance in these clinical strains. Tigecycline and gentamicin retained better activity (resistance ~21 and 11.29%, respectively), while fosfomycin remained highly effective (only 1.61% resistance). In sharp contrast, EOs cassia inhibited 64.51%, cinnamon 56.45%, oregano 33.87%, and thyme 27% of isolates. Fig. 3 illustrates the sensitivity patterns of antibiotics

Table 5. Antibacterial activity of EOs against MRSA

Essential Oil	Number of isolates affected	Percentage affected (%)	Rank (by effectiveness)
Oregano	6	66.6	1 (Most effective)
Cinnamon	4	44.4	2
Cassia	4	44.4	2
Thyme	3	33.3	4
Carom seeds	2	22.2	5 (Least effective)

Table 6. Antibiotic resistance profile against antibiotic discs among *62 ESBL E. coli* isolates.

Bacterial isolates	Antibiotic	Resistance (%)	ZOI Breakpoint (Resistant)
E. coli	Ampicillin	88.70% (55/62)	ZOI < 13 mm
E. coli	Amoxyclav	41.93% (26/62)	ZOI < 13 mm
E. coli	Minocycline	38.70% (24/62)	ZOI < 12 mm
E. coli	Meropenem	29.03% (18/62)	ZOI < 19 mm
E. coli	Tigecycline	20.96% (13/62)	ZOI < 15 mm
E. coli	Gentamicin	11.29% (7/62)	ZOI < 12 mm
E. coli	Cefoxitin	9.67% (6/62)	ZOI < 14 mm
E. coli	Fosfomycin	1.61% (1/62)	ZOI < 12 mm

Table 7. Activity of EOs against *ESBL E. coli. isolates*

Essential oil	Percentage of isolates	Number of isolates
Thyme	27%	17
Oregano	33.87%	21
Cinnamon	56.45%	35
Cassia	64.51%	40
Carom seeds	16.12%	10

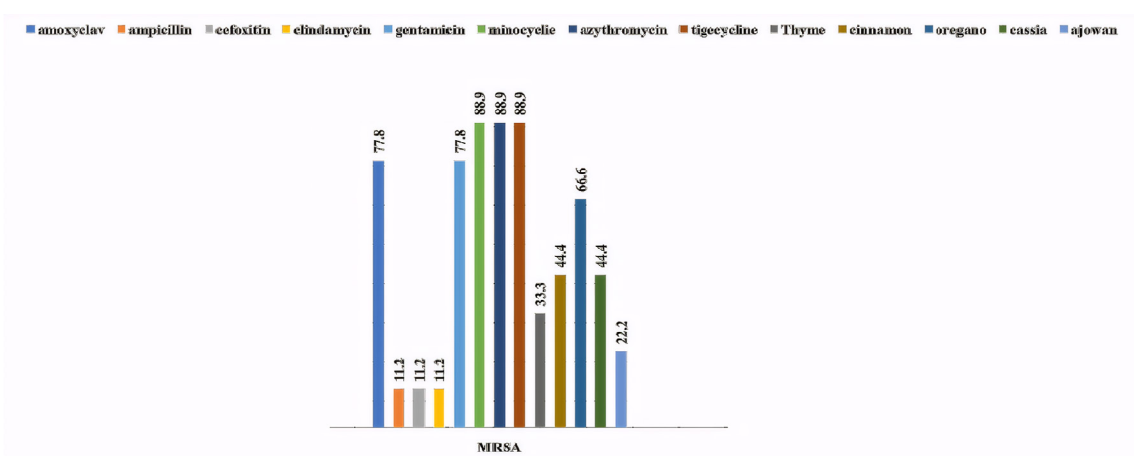

Fig 2. Comparison of the effect of antibiotics and the most effective EOs against MRSA

Table 8. Antibiotic resistance profile against antibiotic discs among 58 field *Staphylococcus spp.* isolates

Bacterial strains	Antibiotic	Resistance percentage	Resistance criterion (ZOI)
Staphylococcus spp	Ampicillin	81.03% (47/58)	ZOI< 28 mm
Staphylococcus spp	Amoxyclav	48.27% (28/58)	ZOI < 19 mm
Staphylococcus spp	Cefoxitin	55.17% (32/58)	ZOI < 21 mm
Staphylococcus spp	Clindamycin	15.51% (9/58)	ZOI < 14 mm
Staphylococcus spp	Gentamicin	20.68% (12/58)	ZOI < 12 mm
Staphylococcus spp	Minocycline	5.17% (3/58)	ZOI < 14 mm
Staphylococcus spp	Azithromycin	20.68% (12/58)	ZOI < 13 mm
Staphylococcus spp	Erythromycin	12.06% (7/58)	ZOI < 13 mm
Staphylococcus spp	Teicoplanin	3.44% (2/58)	ZOI < 10 mm
Staphylococcus spp	Tigecycline	39.65% (23/58)	ZOI < 18 mm

versus EOs against ESBL *E. coli* isolates. Consistent with previous findings (Ginting *et al.*, 2021), cinnamon essential oil exhibits potent antibacterial activity against ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*. It produces large inhibition zones and low minimum inhibitory concentrations (MICs).

Antibiotic resistance profile (per CLSI and EUCAST standards) among the field Staphylococcus spp. Isolates (n=58): Antibiotic resistance profile against antibiotic discs (per CLSI and EUCAST standards) among 58 field *Staphylococcus spp.* isolates is shown in Table 8.

EO sensitivity (strong activity, ZOI > 20 mm) against field Staphylococcus spp. isolates: Activity of EOs against *field Staphylococcus spp. isolates* is shown in Table 9.

Antibiotic susceptibility testing of 58 staphylococcal isolates revealed substantial multidrug resistance, with high rates of ampicillin (81.03%), cefoxitin (55.17%), amoxiclav (48.27%), and tigecycline (39.65%) as per EUCAST criteria. Moderate resistance was seen to gentamicin and azithromycin (both ~20.7%), clindamycin (15.5%), and erythromycin (12.1%), while minocycline (5.17%) and teicoplanin (3.44%) showed lower resistance. This underscores the severe therapeutic challenges posed by resistant staphylococci in clinical settings. In marked contrast, EOs displayed impressive broad-spectrum

Table 9. Activity of EOs against *field Staphylococcus spp. isolates*

Essential Oil	Percentage of isolates	Number of isolates
Oregano	79.31%	46
Cinnamon	68.96%	40
Cassia	67.24%	39
Thyme	65.52%	38
Carom seeds	60.34%	35
Holy basil	3.44%	2
Lemongrass	10.34%	6
Rosewood	3.44%	2
Betel leaf	1.72%	1

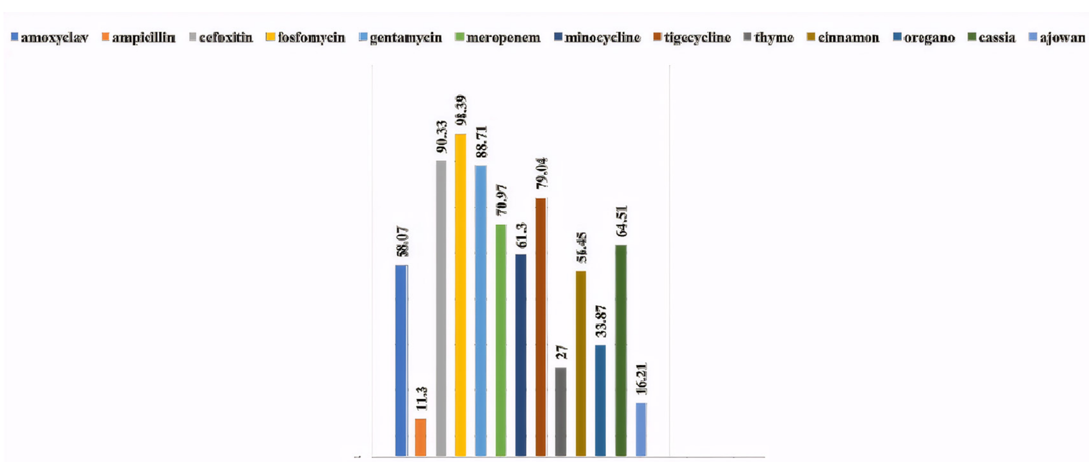

Fig 3. Comparison of effectivity of antibiotics and most effective EOs against ESBL (*Escherichia coli*) isolates

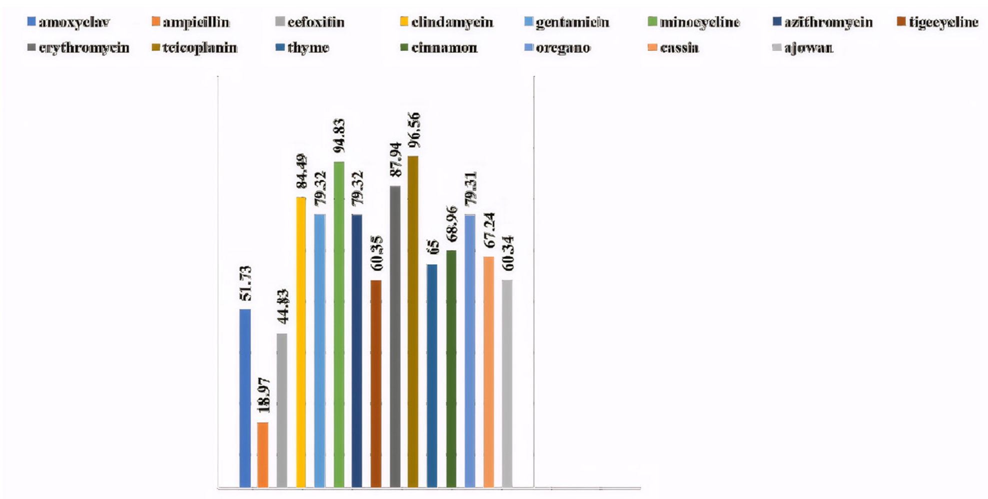

Fig 4. Comparison of effectivity of antibiotics and the most effective EOs against field isolates of *Staphylococcus* spp.

activity against these resistant strains. Oregano inhibited 79.31% of isolates, followed by cinnamon (68.96%), cassia (67.24%), thyme (65.52%) and carom seeds (60.34%). Supporting study confirms that oregano exerts potent antibacterial effects against field clinical staphylococcal isolates, irrespective of their antibiotic sensitivity profiles (Lu *et al*., 2018). Fig. 4 illustrates oregano, cinnamon, cassia, thyme and carom seeds as promising natural alternatives or adjuncts for managing multidrug-resistant staphylococcal infections.

Determination of minimum inhibitory concentration (MIC) for selected EOs: Significant EOs were selected for MIC determination against key bacterial strains using the disc dilution method. Discs impregnated with decreasing EO concentrations showed progressively smaller zones of inhibition. The MIC was defined as the lowest EO concentration producing a visible ZOI > 10 mm.MIC results for the tested EOs are summarized in Table 10.

MIC data across nine bacterial pathogens, including foodborne (*B. cereus, V. parahaemolyticus, L. monocytogenes, Salmonella* spp.) and clinically relevant strains (*E. coli*, ESBL *E. coli*, *K. pneumoniae,S. aureus*, *Y. enterocolitica*), revealed potent antimicrobial activity of several EOs. Oregano exhibited the lowest MIC (0.039 mg/mL against *E. coli*) and strong inhibition (≥0.156 mg/mL) against nine pathogens. Thyme, cassia, lemongrass, and carom seeds also demonstrated broad-spectrum efficacy (MIC≥0.156 mg/mL against 5–7 strains). In contrast, rose, betel leaf, holy basil, and others showed limited potency. Our present findings are substantiated by earlier reports from Oussalah *et al*., 2007 and Mith *et al*., 2014, where oregano, thyme and cassia EOs exhibited consistently low MIC values against foodborne pathogens such as *E. coli*, *Salmonella*, *L. monocytogenes* and *S. aureus*.

Determination of minimum bactericidal concentration (MBC): MBC of the eight most effective EOs was determined against selected bacterial isolates using the microtitre plate method (Fig. 5). Serial two-fold dilutions of each EO were prepared in DMSO and added (20 μL) to 180 μL of standardized overnight-grown bacterial culture in microtitre plate wells. Plates were incubated at 37°C for 24 hours. Following incubation, 100–200 μL aliquots from wells showing the least turbidity were subcultured onto Mueller-Hinton agar (MHA) and incubated for an additional 24 hours at 37°C. The MBC was recorded as the lowest EO concentration that resulted in no visible bacterial growth on the subculture plates (Fig 5). MBC values for the tested EOs are summarized in Table 11.

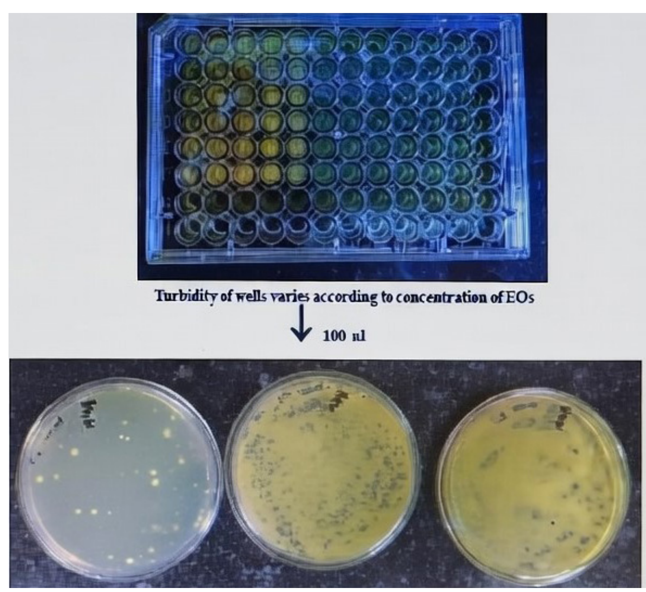

Fig 5. Determination of MBC (Minimum Bactericidal Concentration) by microtitre plate assay

Table 10: Minimum inhibitory concentration (MIC in μL) of selected EOs against different bacterial pathogens

S. No.	Essential Oil	*Bacillus cereus*	*Vibrio parahaemolyticus*	*Listeria monocytogenes*	*Escherichia coli*	*Klebsiella pneumoniae*	*Yersinia enterocolitica*	*Salmonella spp.*	*Staphylococcus aureus*	ESBL *E. coli*
1	Thyme	0.625	0.078	0.3125	0.078	0.156	0.078	0.078	0.156	0.3125
2	Holy basil	0.625	0.625	2.5	1.25	1.25	0.3125	0.625	1.25	2.5
3	Lemongrass	0.156	0.078	1.25	—	5	0.156	0.156	0.625	—
4	Cinnamon	0.625	0.3125	1.25	0.625	0.3125	0.156	0.3125	0.625	1.25
5	Oregano	0.156	0.078	0.3125	0.039	0.078	0.078	0.078	0.156	0.156
6	Rosewood	—	0.3125	—	0.625	0.625	0.156	0.625	—	1.25
7	Cassia	0.156	0.078	0.625	0.3125	0.3125	0.156	0.3125	0.625	0.625
8	Rose	5	1.25	—	2.5	5	5	1.25	5	—
9	Clove	0.625	0.625	2.5	0.625	0.625	0.078	1.25	1.25	1.25
10	Palmarosa	0.625	0.625	5	0.3125	5	0.156	0.3125	1.25	0.156
11	Carom seeds	0.3125	0.156	5	0.3125	0.156	0.078	0.156	0.078	0.625
12	Betel leaf	2.5	1.25	5	0.625	0.625	1.25	0.625	5	5

Table 11. Minimum bactericidal concentration (μL) of EOs against different bacterial strains

S. No	Essential oil	*Bacillus cereus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Yersinia enterocolitica*	*Staphylococcus aureus*	*Salmonella spp.*	*ESBL*
1	Thyme	2.5	0.625	0.3125	0.3125	0.3125	0.3125	1.25
2	Holy basil	2.5	5	2.5	0.625	2.5	2.5	5
3	Cinnamon	1.25	2.5	0.625	0.625	0.625	0.625	2.5
4	Oregano	2.5	0.625	0.156	0.3125	0.3125	0.625	0.625
5	Cassia	2.5	1.25	0.3125	0.3125	1.25	1.25	1.25
6	Clove	5	2.5	1.25	0.3125	1.25	2.5	2.5
7	Carom seeds	5	2.5	0.3125	0.3125	0.625	0.625	2.5
8	Betel leaf	5	5	2.5	0.3125	5	2.5	—

Lower MBC values indicate stronger antibacterial potency. Oregano EO shows the broadest and most potent activity (lowest MBCs: 0.156–0.625 mg/mL against most strains, 0.625 for ESBL). Thyme, cinnamon, cassia, and carom seeds also exhibit strong inhibition (mostly 0.3125–1.25 mg/mL), particularly against Gram-negative pathogens and ESBL strains. Holy basil and betel leaf display weaker activity (higher MBCs, up to 5 mg/mL), while clove and carom seeds perform moderately. These results highlight oregano, thyme, cinnamon, and cassia as the most effective natural antimicrobials among the tested EOs (Mith *et al*., 2014).

Overuse and misuse of antibiotics have rapidly fueled the rise and worldwide dissemination of antimicrobial resistance, leading to significantly higher rates of treatment failure, extended hospital stays, increased mortality, and substantially elevated healthcare expenditures for routine infections such as pneumonia, tuberculosis, bloodstream infections, and gonorrhea (CDC, 2013). Emerging novel resistance mechanisms now jeopardize the successful control of bacterial diseases in both human and animal populations (Hussain *et al*., 2011), underscoring the urgent need for sustainable alternatives to conventional antibiotics in order to preserve therapeutic options and mitigate these escalating public health and economic burdens.

Based on the preceding overview, this study evaluated the antimicrobial potential of 71 Indian EOs against a diverse panel of 144 bacterial isolates, encompassing 15 foodborne pathogens, 9 multidrug-resistant MRSA strains, 62 ESBL-producing *Escherichia coli*, and 58 field-collected *Staphylococcus* spp. Twelve EOs exhibited measurable activity. Notably, thyme, cinnamon, oregano, cassia, and carom seeds consistently produced strong zones of inhibition (ZOI >20 mm) against the majority of tested isolates, while holy basil, lemongrass, rosewood, clove, palmarosa, rose, and betel leaf showed moderate activity (ZOI 10–20 mm). These results are in strong agreement with previous reports demonstrating that phenolic-rich EOs, particularly those dominated by thymol, carvacrol, and cinnamaldehyde, are highly effective against foodborne, spoilage, and multidrug-resistant bacteria through mechanisms involving membrane disruption, leakage of intracellular contents, and inhibition of biofilm formation (Mith *et al*., 2014; Semeniuc *et al*., 2017; Arumugam *et al*., 2016; Ultee *et al*., 2000; Bassolé *et al*., 2010). When applied to meat products, these EOs significantly extend shelf life, substantially lower microbial loads, and show no detectable toxicity. However, their strong aromatic characteristics require thorough sensory assessment before incorporation into food products (Sharma *et al*., 2015, 2017; Kumar *et al*., 2018). Overall, these results underscore the strong potential of EOs from Indian sources as versatile, broad-spectrum natural antimicrobial agents. They offer a viable way to decrease dependence on traditional antibiotics in areas such as clinical medicine, veterinary practice, and food safety. However, their successful real-world adoption will depend on additional refinement, including: Standardization of EO chemical composition, assessment of synergistic blends, comprehensive toxicological and sensory evaluations (Kon and Rai, 2012; Mahboubi and Bidgoli, 2010; Rai *et al*., 2017).

Through assessments of inhibition zones, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC), the research translates lab-based results into potential real-world uses. It emphasizes the promising role of EOs as eco-friendly phytogenic feed additives serving as viable alternatives to conventional antibiotics. These can help lower pathogen burdens, curb the spread of antimicrobial resistance in animal farming systems, improve intestinal health, and contribute to safer food supply chains across poultry, swine, and ruminant production. This aligns with findings from related research (Purkait *et al*., 2020), which supports the antimicrobial and antibiofilm potential of certain EO components in combating resistant bacteria.

Conclusion

This study establishes the significant potential of Essential Oils (EOs) as a sustainable green alternative capable of addressing the global crisis of multidrug resistance in livestock production. Among the 71 EOs screened against 144 multidrug-resistant (MDR) isolates, 12 oils emerged as potent antimicrobial candidates. Specifically, thyme, cinnamon, oregano, cassia, and carom seeds demonstrated consistent broad-spectrum activity, frequently matching or exceeding the efficacy of standard antibiotics against high-priority pathogens like MRSA and ESBL-producing *E. coli*. From a range management perspective, the integration of these plant-derived compounds into livestock systems offers a multi-functional pathway toward sustainability. Their use will reduce pathogens and slow down the development of antimicrobial resistance (AMR) within rangeland-based animal husbandry. By optimizing the rumen fermentation and enhancing nutrient digestibility, enteric methane emissions can potentially be reduced. Further recognizing the biodiversity of rangelands and agroforestry systems as a reservoir for these bioactive compounds aligns with the goals of organic and low-input silvopastoral systems.

While the present study confirms the high potency of select Indian EOs, their successful adoption as phytogenic feed additives requires addressing challenges related to chemical standardization, volatility, and organoleptic impacts on food products. Future research should focus on the synergistic application of these rangeland-derived metabolites and their long-term effects on animal performance in field settings. Ultimately, these findings provide a scientific foundation for utilizing the natural chemical defenses of agroforestry species to safeguard both animal health and environmental integrity in a post-antibiotic era.

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