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Research Article

Antimicrobial Activity of Essential Oils against Potential Food Spoilage Microorganisms

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Abstract | The growing concern about food safety has recently led to the development of natural antimicrobials for control of food borne pathogenic and food spoilage bacteria. Essential oils (EOs) are therefore of great interest as an alternative to conventional food preservatives. The presence of different types of aldehydes, phenolics, terpenes, and other antimicrobial compounds means that the essential oils are effective against a diverse range of pathogens. Present study was conducted to determine the antimicrobial activities of 10 EOs against food-borne pathogenic bacteria and food spoilage bacteria. It was observed that most of the tested EOs exhibit antimicrobial activity against all tested bacteria. Rose oil (Ro) showed the best antimicrobial response. $Gram^+$ bacteria like *Staphylococcus aureus* exhibited more susceptibility than $Gram^-$ bacteria *E. coli* and *P. aeruginosa.* Therefore EOs can be used as additives in food stuff to control food spoilage and food borne pathogenic bacteria.

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Introduction

Food spoilage is a metabolic process caused by a variety of bacteria which brings about changes in sensory properties making food unsuitable or unpleasant for human ingestion resulting in food deterioration and food-borne illnesses (Burkepile *et al.*, 2006). Despite modern advances in food cleanliness, food-borne illnesses remain a major global problem, especially in highly industrialized nations (WHO, 2002). The majority of these food-spoiling microorganisms have the potential to result in unfavourable reactions that can worsen the flavour, odour, colour, sensory, and textural qualities of food while also causing it to spoil. Food must be protected during preparation, storage, and distribution in order for them to have the proper shelf life (Lucera *et al.*, 2012). *Pseudomonas spp*. as well as other gram-negative bacteria like *Enterobacteria* and gram-positive *Staphylococcus spp*. are part of the food spoilage microflora associated with fresh vegetables (Tremonte *et al.*,

2005; Ragaert *et al.*, 2007). Food preservation is the process of defending food against microorganisms and other spoilage factors so that it can last a long time before being consumed. A food's shelf life is the amount of time during which it is stable and conserves the required characteristic. To preserve a food item's nutritional content, texture, and flavour, a change must be made to the product's nature that lowers the microbial load or restricts the proliferation of microorganisms (Amit *et al.*, 2017; Rahman, 2020).

Essential oils (EOs) are the volatile by-products of a plant typically created in specific cells or groups of cells as a means of defence against insects and microbes. They can be produced and stored in secretory cells, cavities, channels, epidermal cells or trichomes in a variety of plant organs, including buds, flowers, leaves, stems, branches, seeds, berries, roots, wood or the bark (Mahato *et al.*, 2019). EOs have long been employed as antimicrobial agents to prevent food spoilage, bacterial growth and illnesses of the digestive tract (Bajpai *et al.*, 2008; Rout *et al.*, 2022). EOs have a strong fragrant aroma with diverse biological, antibacterial, fungicide, larvicidal, analgesic, anti-inflammatory and antioxidant properties (Bakkali *et al.*, 2008; Mendes *et al.*, 2010). EOs stimulate the cell membrane, increasing permeability and allowing important intracellular components to seep out. This also impairs the bacterial enzyme system and cell respiration. By monitoring the efflux of intracellular ions like K^+ and H^+, these effects are analyzed (Van *et al.*, 2010; Pateiro *et al.*, 2021).

Current study was designed to analyze antimicrobial potential of ten essential oils against food spoilage bacteria. The research work was performed in the Immunology Lab, Department of Zoology, Lahore College for Women University, Lahore, Pakistan.

2. Materials and Methods

2.1. Test compound (EOs)

In following study ten EOs were commercially acquired from Aroma Farmacy™ Pakistan. Oils were cardamom, lemon grass, peppermint, rose, rosemary, patchouli, ginger, eucalyptus, orange and lemon. The samples were kept at room temperature until further analysis.

2.2. Bacterial Strains

Clinical bacterial isolates were obtained from Services Institute of Medical Sciences (SIMS), Lahore. Total 5 strains were collected, out of those, 3 strains were selected, two Gram negative (G^-) strains (*Escherichia coli*, *Pseudomonas aeruginosa*) and one Gram positive (G^+) strain (*Staphylococcus aureus*). Bacterial sub-cultures were prepared on nutrient agar slants then stored in refrigerator at 4ºC till further use.

2.3. Antimicrobial Activity

Four different methods with modified protocols were used for the antimicrobial assay of EOs i.e., well diffusion method (Rivera *et al.*, 2023), disc diffusion method (Hudzicki, 2009), MIC broth dilution method (Wiegand *et al.*, 2008) and antibiotic sensitivity test (Ericsson and Sherris, 1971).

3. Results

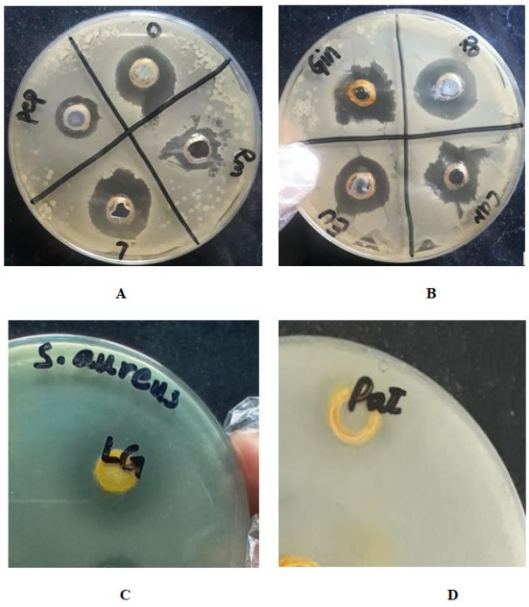
3.1. Well diffusion method

Approximately 10µl of each EO was loaded in 6mm agar boreholes made in agar media plates inoculated with bacterial cultures. Plates were incubated for 12 hours after that the diameters of the zones of inhibition were measured. A comparison of antimicrobial activity of EOs against selected food spoilage bacteria is provided in Tables 1, 2 and 3.

For *S. aureus* the largest zone of inhibition (24±0.8mm) was observed against lemon grass, whereas the least antimicrobial activity, hence the smallest zone (11±0.7mm) was observed against patchouli (Table 1 and Figure 1).

Table 1: Antimicrobial activity exhibited by well diffusion assay of EOs against *S. aureus*

Sr.	Essential Oils	Zone of Inhibition (mm)
1.	Cardamom	18±0.8
2.	Eucalyptus	20±0.7
3.	Lemon	21±0.8
4.	Orange	20±0.7
5.	Patchouli	11±0.7
6.	Rosemary	15±0.7
7.	Ginger	21±0.8
8.	Rose Oil	23±0.5
9.	Peppermint	14±0.7
10.	Lemon Grass	24±0.8



A B C D

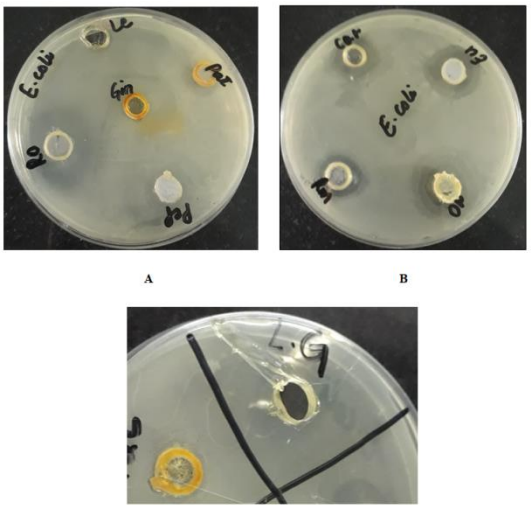
*O: Orange, Pep: Peppermint, Rm: Rosemary, L: Lemon, Gin: Ginger, Ro: Rose oil, Car: Cardamom, Eu: Eucalyptus, LG: Lemon Grass, Pat: Patchouli

Figure 1: Antimicrobial activity of EOs against *S. aureus*

For *E. coli* the largest zone of inhibition (24±0.5mm) was observed against rose oil, whereas the least antimicrobial activity, hence the smallest zone (11±0.7mm) was observed against ginger (Table 2 and Figure 2).

Table 2: Antimicrobial activity exhibited by well diffusion assay of EOs against *E. coli*

Sr.	Essential Oils	Zone of Inhibition (mm)
1.	Cardamom	16±0.7
2.	Eucalyptus	20±0.6
3.	Lemon	13±0.8
4.	Orange	13±0.7
5.	Patchouli	13±0.8
6.	Rosemary	14±0.8
7.	Ginger	11±0.7
8.	Rose Oil	24±0.5
9.	Peppermint	13±0.8
10.	Lemon Grass	15±0.2



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*O: Orange, Pep: Peppermint, Rm: Rosemary, L: Lemon, Gin: Ginger, Ro: Rose oil, Car: Cardamom, Eu: Eucalyptus, LG: Lemon Grass, Pat: Patchouli

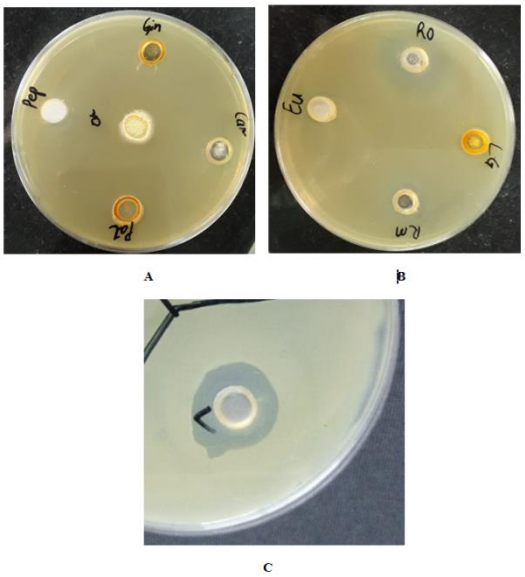
Figure 2: Antimicrobial activity of EOs against *E. coli*

Table 3: Antimicrobial activity exhibited by well diffusion assay of EOs against *P. aeruginosa*

Sr.	Essential Oils	Zone of Inhibition (mm)
1.	Cardamom	0±0
2.	Eucalyptus	10±0.8
3.	Lemon	14±0.5
4.	Orange	13±0.9
5.	Patchouli	0±0
6.	Rosemary	13±0.8
7.	Ginger	0±0
8.	Rose Oil	19±0.5
9.	Peppermint	0±0
10.	Lemon Grass	11±0.3

For *P. aeruginosa* the largest zone of

inhibition (19±0.5mm) was observed against rose oil, whereas cardamom, patchouli, ginger and peppermint EOs didn't exhibit any antimicrobial activity (Table 3 and Figure 3).



*O: Orange, Pep: Peppermint, Rm: Rosemary, L: Lemon, Gin: Ginger, Ro: Rose oil, Car: Cardamom, Eu: Eucalyptus, LG: Lemon Grass, Pat: Patchouli

Figure 3: Antimicrobial activity of EOs against *P. aeruginosa*

A comparison of antimicrobial activity of EOs against *S. aureus*, *E. coli* and *P. aeruginosa* with well diffusion method is provided in Table 4.

Table 4: A comparison of antimicrobial activity of EOs by well diffusion method

Essential Oils	Bacterial Strains Zone of Inhibition (mm)		
	S. aureus	***E. coli***	***P. aeruginosa***
Cardamom	18±0.8	16±0.7	0±0
Eucalyptus	20±0.7	20±0.6	10±0.8
Lemon	21±0.8	13±0.8	14±0.5
Orange	20±0.7	13±0.7	13±0.9
Patchouli	11±0.7	13±0.8	0±0
Rosemary	15±0.7	14±0.8	13±0.8
Ginger	21±0.8	11±0.7	0±0
Rose Oil	23±0.5	24±0.5	19±0.5
Peppermint	14±0.7	13±0.8	0±0
Lemon Grass	24±0.8	15±0.2	11±0.3

3.2. Disc diffusion method

The antimicrobial activity of EOs was evaluated by disc diffusion method. Agar media plates were inoculated with bacterial strains and EOs impregnated filter paper discs of 5mm were placed on the agar plates. Plates were incubated for 12 hours, after that the diameters of the zones of inhibition were measured. A comparison of antimicrobial activity of EOs against selected food spoilage bacteria by disc diffusion method is provided in Tables 5, 6 and 7.

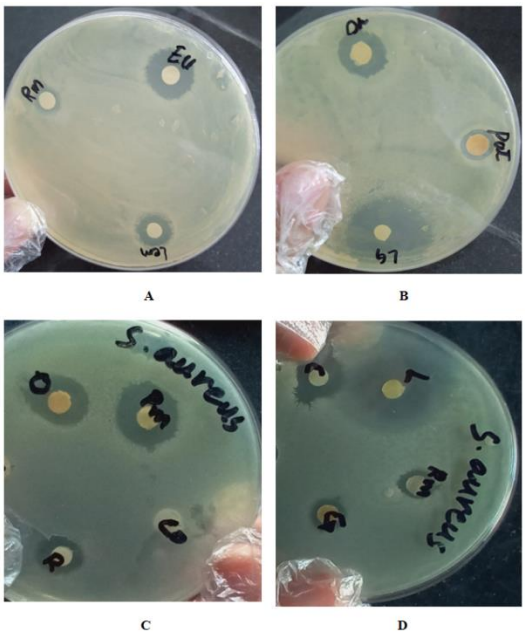
For *S. aureus* the largest zone of inhibition (28±0.4mm) by disc diffusion method was observed against lemon grass, whereas the least antimicrobial activity, hence the smallest zone (8±0.2mm) was observed against rosemary (Table 5 and Figure 4).

Table 5: Antimicrobial activity of EOs evaluated by disc diffusion method against *S. aureus*

Sr.	Essential Oils	Zone of Inhibition (mm)
1.	Cardamom	12±0.8
2.	Eucalyptus	15±0.7
3.	Lemon	12±0.8
4.	Orange	19±0.4
5.	Patchouli	9±0.7
6.	Rosemary	8±0.2
7.	Ginger	10±0.7
8.	Rose Oil	20±0.7
9.	Peppermint	10±0.8
10.	Lemon Grass	28±0.4

Table 6: Antimicrobial activity of EOs evaluated by disc diffusion method against *E. coli*

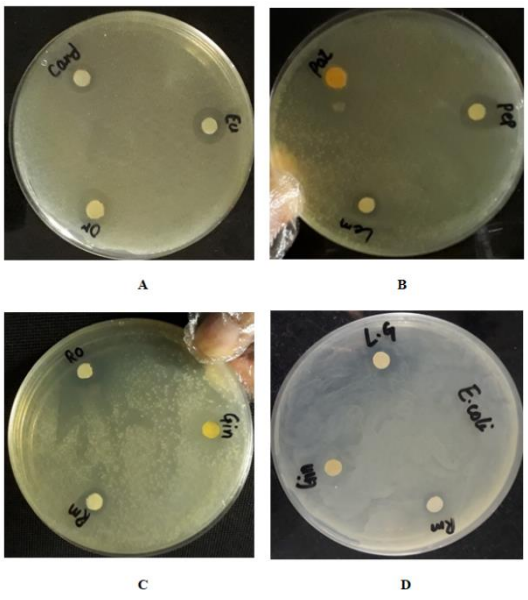
Sr.	Essential Oils	Zone of Inhibition (mm)
1.	Cardamom	10±0.8
2.	Eucalyptus	14±0.7
3.	Lemon	10±0.3
4.	Orange	12±0.4
5.	Patchouli	8±0.6
6.	Rosemary	10±0.2
7.	Ginger	13±0.7
8.	Rose Oil	11±0.7
9.	Peppermint	10±0.8
10.	Lemon Grass	10±0.4



*O: Orange, Pep: Peppermint, Rm: Rosemary, L: Lemon, Gin: Ginger, Ro: Rose oil, Car: Cardamom, Eu: Eucalyptus, LG: Lemon Grass, Pat: Patchouli

Figure 4: Antimicrobial activity of EOs against *S. aureus* by disc diffusion method

For *E. coli* the largest zone of inhibition (14±0.7mm) by disc diffusion method was observed against eucalyptus, whereas the least antimicrobial activity, hence the smallest zone (8±0.6mm) was observed against patchouli (Table 6 and Figure 5).



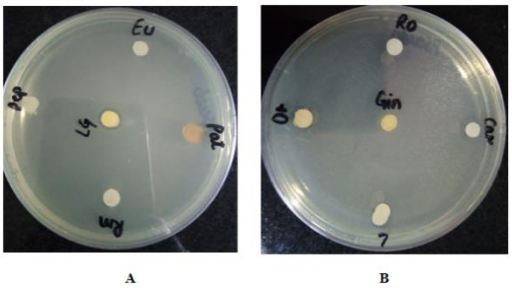
*O: Orange, Pep: Peppermint, Rm: Rosemary, L: Lemon, Gin: Ginger, Ro: Rose oil, Car: Cardamom, Eu: Eucalyptus, LG: Lemon Grass, Pat: Patchouli

Figure 5: Antimicrobial activity of EOs against *E. coli* by disc diffusion method

For *P. aeruginosa* the largest zone of inhibition (7±0.3mm) by disc diffusion method was observed against lemon, whereas the no antimicrobial activity was observed against patchouli, rosemary, ginger and peppermint (Table 7 and Figure 6).

Table 7: Antimicrobial activity of EOs evaluated by disc diffusion method against *P. aeruginosa*

Sr.	Essential Oils	Zone of Inhibition (mm)
1.	Cardamom	8±0.8
2.	Eucalyptus	7±0.7
3.	Lemon	7±0.3
4.	Orange	9±0.4
5.	Patchouli	0±0
6.	Rosemary	0±0
7.	Ginger	0±0
8.	Rose Oil	9±0.7
9.	Peppermint	0±0
10.	Lemon Grass	7±0.4



*O: Orange, Pep: Peppermint, Rm: Rosemary, L: Lemon, Gin: Ginger, Ro: Rose oil, Car: Cardamom, Eu: Eucalyptus, LG: Lemon Grass, Pat: Patchouli

Figure 6: Antimicrobial activity of EOs against *P. aeruginosa* by disc diffusion method

A comparison of antimicrobial activity of EOs against *S. aureus*, *E. coli* and *P. aeruginosa* by disc diffusion method is provided in Table 8.

Table 8: A comparison of antimicrobial activity of EOs by disc diffusion method

Essential Oils	Bacterial Strains Zone of Inhibition (mm)		
	S. aureus	*E. coli*	*P. aeruginosa*
Cardamom	12±0.8	10±0.8	8±0.8
Eucalyptus	15±0.7	14±0.7	7±0.7
Lemon	12±0.8	10±0.3	7±0.3
Orange	19±0.4	12±0.4	9±0.4
Patchouli	9±0.7	8±0.6	0±0
Rosemary	8±0.2	10±0.2	0±0
Ginger	10±0.7	13±0.7	0±0
Rose Oil	20±0.7	11±0.7	9±0.7
Peppermint	10±0.8	10±0.8	0±0
Lemon Grass	28±0.4	10±0.4	7±0.4

3.3. Antibiotic sensitivity test

The EOs were evaluated in comparison with three different antibiotics (Erythromycin, Streptomycin and Ampicillin). Four discs on a single agar media plate were applied three of them were antibiotic discs and fourth one was EOs impregnated filter paper discs. Plates were incubated for 12 hours, after that the diameters of the zones of inhibition were measured. The antibiotic sensitivity test activity of 10 EOs against selected food spoilage bacteria is provided in a graphical representation in Table 9.

Table 9: Zones of inhibition (mm) exhibited due to antibacterial activity of EOs in comparison with antibiotics against selected bacterial strains

Bacterial Strains	*S. aureus*	*E. coli*	*P. aeruginosa*
EOs			
Car	14±0.8	12±0.5	0
Eu	12±0.7	13±0.8	11±0.7
Le	12±0.8	10±0.4	9±0.4
Or	12±0.8	11±0.7	11±0.6
Pat	11±0.4	13±0.5	0
Rm	7±0.7	10±0.7	11±0.4
Gin	9±0.4	14±0.8	0
Ro	15±0.9	16±0.7	11±0.5
Pep	10±0.5	7±0.5	0
LG	15±0.6	14±0.8	8±0.3
Antibiotics			
E	22±0.9	34±0.7	26±0.6
S	17±0.7	23±0.8	20±0.7
AMP	10±0.8	9±0.7	0

*E: Erythromycin, S: Streptomycin, Amp: Ampicillin, Car: Cardamom, Eu: Eucalyptus, Le: Lemon, Or: Orange, Pat: Patchouli, Rm: Rosemary, Gin: Ginger, Ro: Rose oil, Pep: Peppermint, LG: Lemon grass

The most antibiotic activity and hence the largest zone of inhibition (16±0.7) was observed in rose oil against *E. coli*, whereas the least antibiotic activity and hence the smallest zone of inhibition (7±0.5) was observed in peppermint against *E. coli*. The cardamom, patchouli, ginger and peppermint exhibited no antibiotic activity against *P. aeruginosa*, however these EOs exhibited moderate to high antibiotic effects against *S. aureus* (14±0.8, 11±0.4, 9±0.4, 10±0.5) and *E. coli* (12±0.5, 13±0.5, 14±0.8, 7±0.5).

3.4. Minimum inhibitory concentration

MIC was determined by broth dilution method. Three EOs (rose oil, lemon grass and ginger) were selected and diluted in 3ml broth. Various concentrations of EOs (25µl/ml, 50µl/ml, 75µl/ml and 100µl/ml) were incubated with the bacterial cultures then measured spectrophotometrically at 600nm. The percentage of growth inhibition is provided in Table 10.

Table 10: Determination of MIC of EOs against selected bacterial strains

Concentrations of EOs	Essential Oils		
	Ro	LG	Gin
	S. aureus		
25µl/ml	42%	2.5%	10%
50µl/ml	52%	3.9%	31%
75µl/ml	63%	4.4%	37%
100µl/ml	81%	7.8%	40%
	E. coli		
25µl/ml	5.7%	34%	6.7%
50µl/ml	10%	63%	7%
75µl/ml	18%	86%	35%
100µl/ml	51%	88%	60%
	P. aeruginosa		
25µl/ml	11%	9%	4%
50µl/ml	45%	11%	7%
75µl/ml	54%	13%	9%
100µl/ml	68%	18%	13%

4. Discussion

The present study was undertaken to

estimate the antimicrobial activity of EOs against potential food spoilage microorganisms. Antimicrobial potency of EOs was evaluated against clinical isolates of *S. aureus*, *E. coli* and *P. aeruginosa*. The effects of EOs on bacterial growth were studied by observing zones of inhibition by well diffusion method against Gram$^+$ *S. aureus*. It was observed that the zone appeared in all of the EOs. The maximum zone was exhibited by LG (24mm) followed by Ro (23mm) and Le (20mm). The least zone was exhibited by Pat (11mm). Against Gram$^-$ *E. coli* all tested EOs exhibited zones of inhibition, the maximum zone was observed for Ro (24mm) followed by Eu (20mm) and the least zones were for Pep, Le, Or and Pat. The EOs tested against Gram$^-$ *P. aeruginosa* showed moderate to no antimicrobial activity, the maximum zone was observed by Ro (24mm) followed by Le (14mm), LG (12mm) and Eu (10mm) while Car, Gin, Pep and Pat show no activity. These findings are in agreement with similar results reported by Aiemsaard *et al*. (2011) on *S. aureus* against *Cymbopogon citratus*. Shohayeb *et al*. (2014) described similar results on *S. aureus* against *Rosa damascene*. Fisher and Phillips (2006) studied the antimicrobial effects of *S. aureus* against *Citrus limon* and concluded in agreement with results of resent work. The comparison of all tested EOs against three bacteria showed that the Gram$^+$ bacteria were more susceptible to EOs than Gram$^-$ bacteria. Similarly, Bosnic *et al*. (2006) analyzed the antimicrobial activity of rosemary and eucalyptus EOs against *S. aureus*, *B. subtillis* and *P. aeruginosa* by well diffusion method and concluded in accordance with findings of present work.

The effect of essential oil on bacterial growth was studied by disc diffusion method against S. *aureus*, it was observed that LG had the maximum zone (28mm) followed by Ro (22mm) and the least zone was observed for Rm (8mm). The activity against *E. coli* showed maximum zone by Eu (14mm) and least for Pat, whereas for *P. aeruginosa* the maximum zone was observed by Ro (9mm) and least by LG (7mm). Similar results were reported by several workers (Vaishali and Geetha, 2018; Sechi *et al*., 2001; Balhaddad, and AlSheikh, 2023).

MIC showed that the maximum growth inhibition was observed for Ro (86%) followed by Gin (40%) and LG (4.4%) against *S. aureus*. The MIC was the highest for LG (88%) followed by Gin (60%) and Ro (51%) against *E. coli*, whereas the MIC was the highest for LG (88%) followed by Gin (60%) and Ro (51%) against *P. aeruginosa*. Similar to present study Nagalakshami *et al*., (2019) analyzed the EOs against two Gram$^+$ bacteria *S. aureus* and *B. subtilis* and obtained similar percentages for growth inhibition. Moreira *et al*. (2005) assayed strains of *E. coli* which exhibited similar susceptibilities to the action of the EOs.

5. Conclusion

It was concluded that most of the tested essential oils exhibited antimicrobial activity against all tested bacteria. Ro shows the overall maximum antimicrobial effect. The Gram$^+$ bacteria *S. aureus* exhibited more susceptibility than the Gram$^-$ bacteria *E. coli* and *P. aeruginosa.* EOs can be used as additives in foods to control food spoilage and food born pathogenic bacteria.

6. Acknowledgments

All research work was performed entirely in the Immunology Lab, Department of Zoology, Lahore College for Women University, Lahore.

7. Author's Contribution

Both authors have contributed equally towards the performance of the lab work and manuscript write-up.

8. Conflict of Interest

Authors have declared no Conflict of Interest

9. References

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