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Exploring the Antimicrobial Properties of Lemon: A Comparative Analysis of Peel, Seed, and Pulp

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Lemons are a treasure trove in nature, belonging to the Rutaceae family and rich in vitamin C, as well as various macro and micronutrients. They are widely known for boosting immunity and can potentially be used as a natural source of medication. D-limonene is one of the main bioactive compounds present in lemons, and it is responsible for the refreshing fragrance of lemons. The study aims to identify whether the waste from lemons can be used as potential nutraceuticals or functional foods

Study Design: We took five different species for comparative antibacterial studies from Citrus species those are *C. aurantifolia, C. limetta, C. sinensis, C.reticulata, and C. maxima.*

Place and Duration of Study: Biotechnology lab, Techno India University, Kolkata. The duration of this study was 1 year.

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Methodology: In this comparative study, the major parts (pulp, seed, and peel) of a fruit were used for the experiments. It is important to reduce the amount of waste in the environment by creating creative and cost-effective, eco-friendly waste management techniques. The antibacterial potential against E. coli and S. aureus was thoroughly measured using the Kirby-Bauer disc diffusion method.
Results: The results show that in the case of *E. coli* inhibition , the peels of *C. reticulate* (58.33 ±0.4 mm), the seeds of *C. aurantifolia* (58.33 ±0.4 mm), and the pulp of *C. aurantifolia* (55.33 ±2.94 mm) perform well compared to other samples. In the case of *S. aureus* inhibition, the peels of *C. limetta* (51±1.41mm), the seeds of *C. aurantiifolia* (58.33 ±0.4 mm), and the pulp of *C. aurantifolia* (49±2.82mm) perform well compared to other samples.
Conclusion: Hence, the results indicate that PEELs can be potential antimicrobial agents and have discovered that various parts of the citrus fruit exhibit a wide range of antimicrobial effects against both gram-positive and gram-negative bacteria.

Keywords: Citrus peel; seed; pulp; antimicrobial; citrus fruits.

1. INTRODUCTION

Citrus fruit belongs to the Rutaceae family. They are rich in citric acid and ascorbic acid, strengthening our immune system .Citrus fruits are one of the most widely grown fruits and are an important source of physiologically active substances and phytochemicals [1]. They can potentially be employed as a natural medication source or a major component of functional food since they are an excellent source of bioactive compounds and are high in ascorbic and citric acid, which boosts our immune systems. These compounds are mostly recognized in their edible parts. On the other hand, limonoids—typical citrus fruit triterpenoids with an intensely bitter taste and potentially anticarcinogenic and chemo-preventive properties can be extracted from seeds [2].

Citrus fruits increase waste generation when consumed raw or juiced. Conventional rubbish disposal techniques contaminate land and waterways, potentially harming aquatic environments .Consequently, to lessen the quantity of waste that builds up In the environment, it is essential to create creative processes and economical, ecologically friendly waste management techniques [3].

Bacterial infections worldwide are a leading cause of illnesses, physical impairments, and deaths. Medicinal plants are thought to offer a safer and more affordable alternative for treating bacterial infections because they contain a diverse array of phytochemicals. Natural medicines made from medicinal plants have antibacterial properties that can be used to treat viral, fungus, and bacterial illnesses. Microorganisms are becoming more resistant to antibiotics, despite the pharmaceutical industry having developed several new ones during the past three decades [4]. Many years of antibacterial study have been conducted on citrus peels, pulp, and seeds, and it has been demonstrated that seed wastes consist of a substantial number of essential oils and polyphenols with antimicrobial activity. Bioactive chemicals [5] with potential medicinal applications can be found in abundance in citrus trash. Numerous advantageous characteristics of these compounds have been discovered, such as their anti-aging, anti-mutagenic, anti-carcinogenic, and anti-allergenic effects. One particularly interesting source of natural chemicals with potential for therapeutic use is *Citrus sinensis*. New medications may therefore be developed as a result of more research into the extraction and application of these substances from citrus trash [6].

In this current study the antibacterial properties of citrus seed, peel, and pulp extracts from *Citrus reticulata*, *Citrus limetta*, *Citrus aurantifolia*, *Citrus sinensis*, and *Citrus maxima* and d-limonene (essential oil rich in terpenes found in the rind of citrus fruits) were investigated using the agar-well diffusion methodagainst *Staphylococcus aureus* and *Escherichia coli* a gram-positive and a gram-negative bacteria respectively.

1.1 Literature Study

Seeds were found to contain alkaloids, saponins, and other compounds that contribute to their antimicrobial activity. The antimicrobial effects of *Citrus limetta* were also highlighted, attributed to the presence of limonene and other compounds [7]. The essential oil exhibited dose-dependent activity against S. aureus, more so than against E. coli, according to the results of the antibacterial activity analysis. In comparison to ampicillin, the positive control, its antibacterial activity is significantly lower [8].

According to the results of the antibacterial activity analysis, the essential oil exhibits dose-dependent activity against S. aureus more so than it does against E. coli. In contrast to ampicillin, the positive control,its antibacterial activity is significantly lower [9].

The study revealed that extracts from citrus fruits, specifically *Citrus reticulata* and *Citrus limetta*, have demonstrated antibacterial properties against certain bacterial strains. *Citrus reticulata* peel extract was more effective against gram-positive bacteria, particularly showing a high inhibition zone against Klebsiella pneumoniae. On the other hand, *Citrus limetta* peel extract was found to have superior antibacterial effectiveness against specific bacterial strains compared to its juice extract, especially in inhibiting gram-positive bacteria like Bacillus sp. However, *Citrus limetta* juice extract was more efficient than its peel extract against gram-negative bacteria. It's worth noting that 10% DMSO did not exhibit any inhibition zone. In the case of *Citrus maxima*, its peel extract showed stronger antibacterial action against Bacillus sp among gram-positive bacteria, while its juice extract was more effective against S. aureus and had a maximum inhibition zone against E. coli. The study also found that the juice extract of *Citrus maxima* was more efficient against gram-negative bacteria than its peel extract [10].

Citrus aurantifolia is rich in phytochemical components such as flavonoids alkaloids tannins and phenols all of which have been shown to have antibacterial qualities [11]. It was found that ethanolic extract of pomelo (*Citrus maxima*) seeds and pulp exhibited the largest zones of inhibition for Staphylococcus aureus. The ethanolic extract of pomelo (*Citrus maxima*) seeds and pulp showed low inhibition zone against Escherichia coli and Bacillus subtilis compared to ethanolic extract of grapefruit (*Citrus paradisi*) seeds and pulp. Ethanolic extract of grapefruit (*Citrus paradisi*) seeds and pulp showed inhibition zones more for Staphylococcus aureus compared to Bacillus subtilis Escherichia coli [12]. The inhibition zones for the Bacillus subtilis in the investigated pomelo (*Citrus maxima*) seeds and pulp ethanolic extract is higher than the grapefruit seeds extract sample [13].

It was identified that beneficial antibacterial effect has contributed to the antimicrobial activity of citrus flavonoids, such as naringenin and hesperidine. The research also showed clear disparity between the antimicrobial activity of the pomelo (*Citrus maxima*) seeds and pulp ethanolic extract and the grapefruit (*Citrus paradisi*) seeds and pulp ethanolic extract [14].

In the study, it was found that pulp has more antioxidant activity and antimicrobial activity as compare with the orange peel [15]. It was also observed that limonene showed a significant antibacterial activity in the growth and reproduction of S. aureus. The number of colonies lowered gradually with the increased concentration of limonene [16]. It was determined that the antibiotic potential and antibacterial activities of lemon is significantly higher against drug-resistant phenotypes compared to other fruits [17].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection of samples

Sample collection was done from local vendors in Kolkata, West Bengal between August 2023 and October 2023 throughout the year. The collected sample of seeds, peels, and pulps were then powdered in a hot air oven. According to Sulaiman et al., It is best to limit the interval between sample harvest and experimental work, as dried samples are easier to work with than fresh samples, which are difficult to handle and tend to disintegrate easily [18]. Oven drying that uses thermal energy to eradicate moisture from samples is considered an easy and rapid process that preserves phytochemicals and essential antioxidants as mentioned by [19]. D-limonene source from Sigma-Aldrich

2.1.2 Microorganisms

Staphylococcus aureus and Escherichia coli bacterial strains were obtained from the Department of Biotechnology, Techno India University, West Bengal, Salt Lake, Kolkata-700091. The bacteria strains were grown in Himedia M002-100G Nutrient broth at 37^0 Celsius or 98.6 Fahrenheit in a shaker incubator overnight.

2.1.3 Sample preparation

The dehydrated samples were then powdered finely using a household blender and 1 g of every sample was then added to 20 ml of double distilled water to make a final concentration of 50 mg/ml. After thoroughly mixing the mixture, it was left in the dark for an entire night on a vibration table set to a low setting. After passing the mixture through the Whatman No. 1 filter paper, the aqueous extract was obtained and used without any additives.

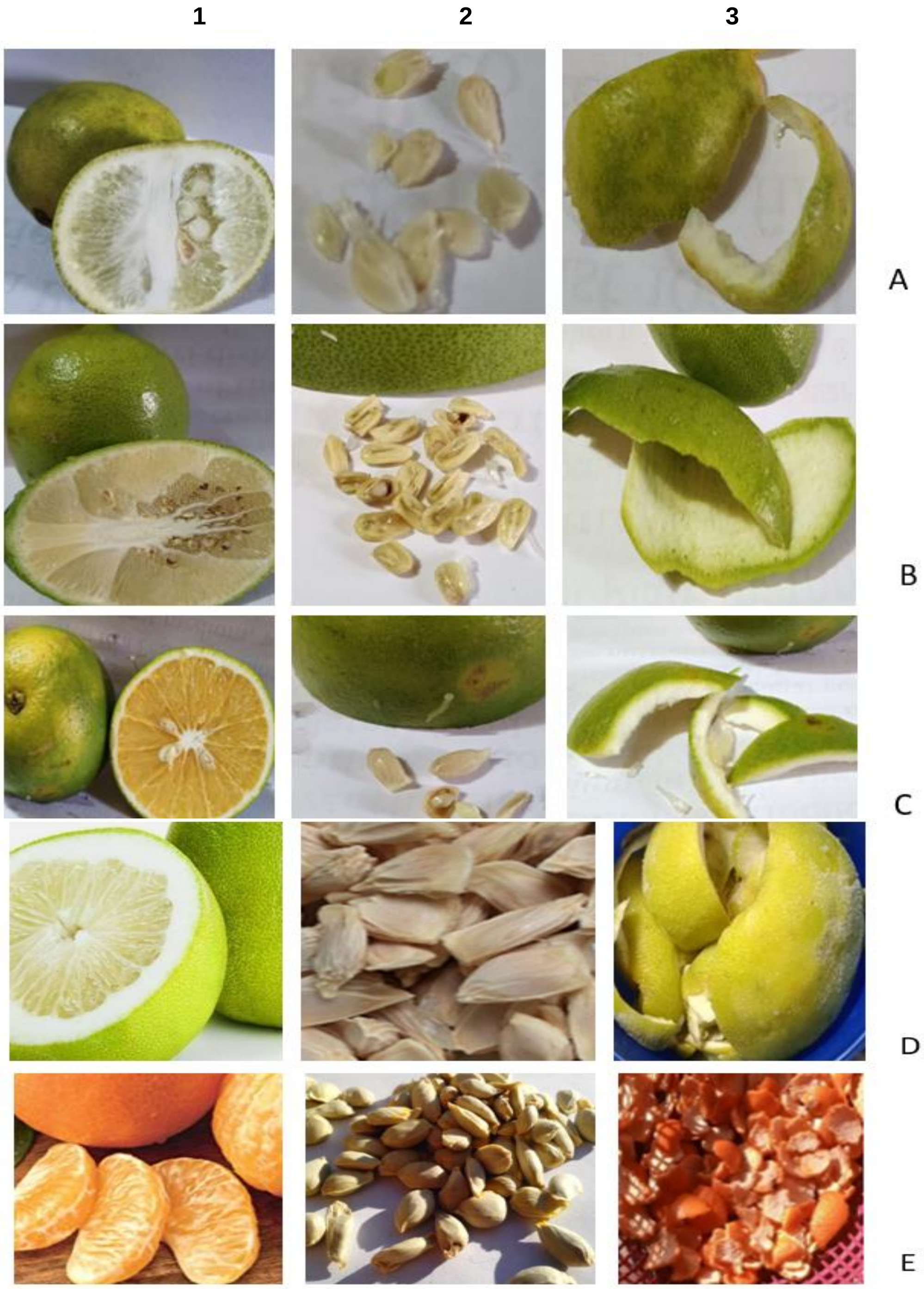

Image 1. The samples are A – *Citrus aurantifolia*, B- *Citrus reticulata*, C – *Citrus limetta*, D- *Citrus maxima*, E- *Citrus sinensis*. 1 denotes pulp, 2 denotes seed and 3 denotes peel of the above-mentioned samples

2.2 Methodology

The Kirby-Bauer test was used to assess antibiotic susceptibility. Extracts and d-limonene were tested using the disc diffusion method on agar plates. Samples were added to filter paper discs and placed on bacterial plates, which were then incubated to observe the zones of inhibition.

3. RESULTS AND DISCUSSION

The seeds, pulp, and peel of a total of 5 commonly found citrus species samples were taken namely *Citrus reticulata*, *Citrus limetta*, *Citrus aurantifolia*, *Citrus sinensis*, and *Citrus maxima* along with d-limonene, a vital essential oil found in every citrus species. Water extracts of the samples were subjected to the Kirby-Bauer test for antibiotic susceptibility. We chose two different bacteria, Staphylococcus *aureus,*and Escherichia *coli* which are gram-positive and gram-negative respectively. Different concentrations of ampicillin were also subjected to the test to compare the efficiency of the samples.

3.1 Antimicrobial Efficacy of Samples against Gram-negative Bacteria Escherichia *coli*

Different concentrations of **Ampicillin** have shown different inhibitory zones on *E. coli*. Every time 40 µl of the sample was used for the treatment 0.4 mg 64 ± 2.44 mm, 0.2 mg - 63 ± 2.5 mm, 0.1 – 53.66 ±1.6 mm, 0.05 mg - 50 ±1.5 mm, 0.025mg – 40 ±1.5 mm [Fig. 2].

The result shows *C. aurantiifolia* and *C.maxima* have the highest antimicrobial activity among other samples. Sd measured (n=3) for each sample [Fig. 3] Pictures show the zones in which the samples have inhibited bacterial growth. Seed extracts of *C. aurantiifolia* (58.33 ±0.4 mm) and *C. maxima* (57 ±3.5 mm) showed good antibacterial efficacy [Fig. 6].

C. reticulata and *C.sinensis* have the highest antimicrobial activity among other samples. Sd measured (n=3) for each sample [Fig. 4] Pictures show the zones in which the samples have inhibited bacterial growth. Peel extracts of *C. reticulate* (58.33 ±0.4 mm) and *C. sinensis* (57 ±3.5 mm) showed good antibacterial efficacy.

The result shows *C. aurantiifolia* and *C.maxima* have the highest antimicrobial activity among other samples. Sd measured (n=3) for each sample [Fig. 5] Pictures show the zones in which the samples have inhibited bacterial growth. Pulp extracts of *C. aurantiifolia* (55.33 ±2.94 mm) *and C. reticulata* (50±2.6 mm) showed good antibacterial efficacy [Fig. 6].

Every time 40 µl of the sample has been used for the treatment 0.4 mg 65± 2.44 mm, 0.2 mg - 63 ± 2.5 mm, 0.1 – 53.66 ±1.6 mm, 0.05 mg - 30.5 ±1.5 mm, 0.025mg – 20 ±1.5 mm [Figs. 7, 8]

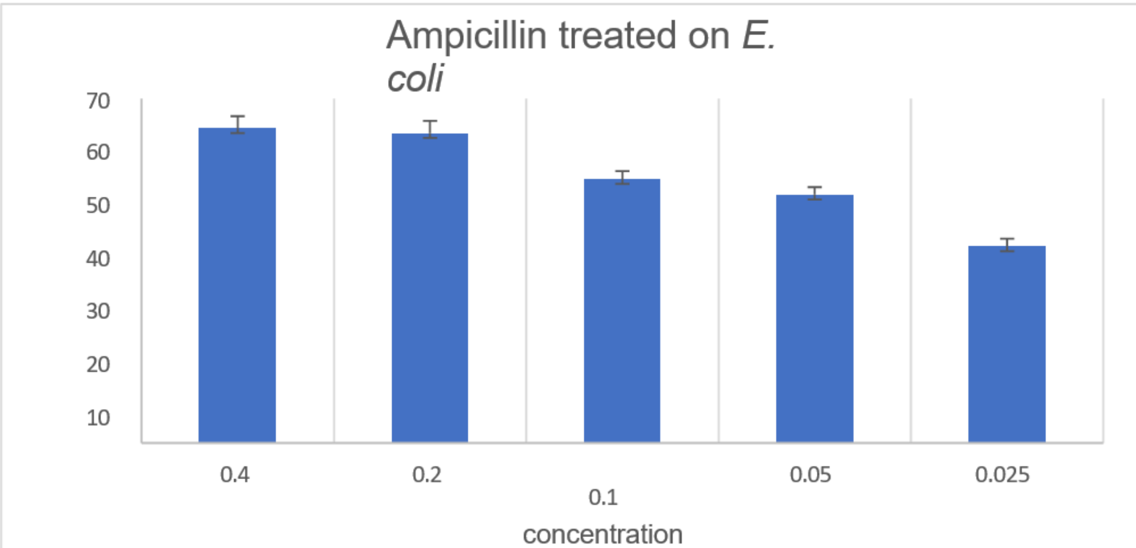

Fig. 1. Diagrammatic representation of the antimicrobial activity of Ampicillin on *E. coli* bacteria

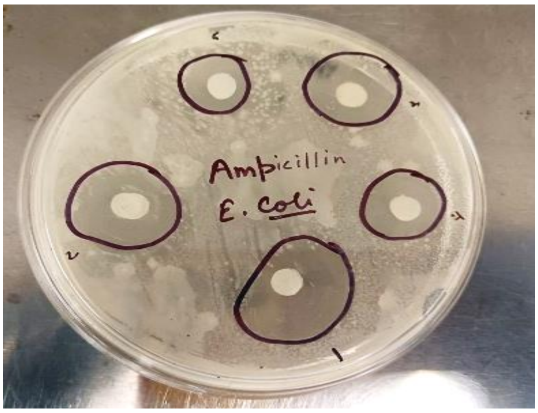

Fig. 2. A pictorial representation of the inhibitory zones of ampicillin

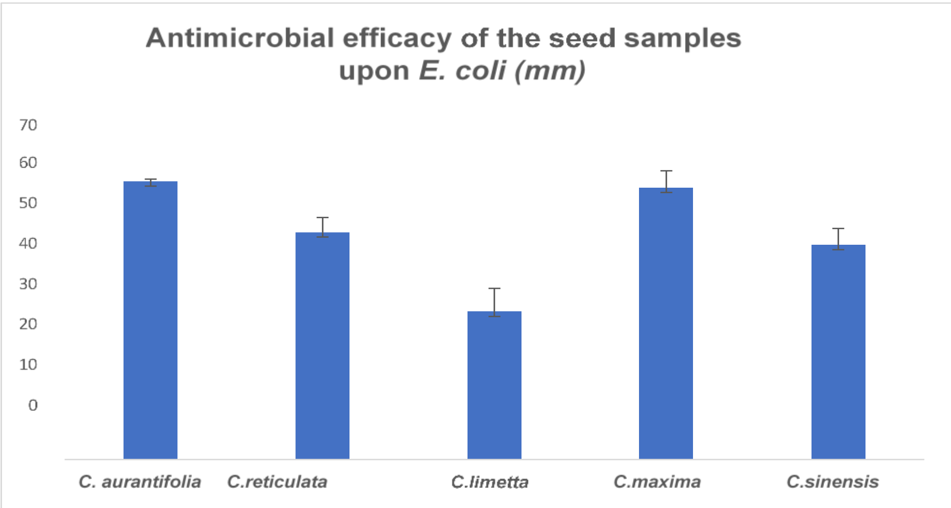

Fig. 3. Diagrammatic representation of the antimicrobial activity of the seed samples on *E. coli* bacteria

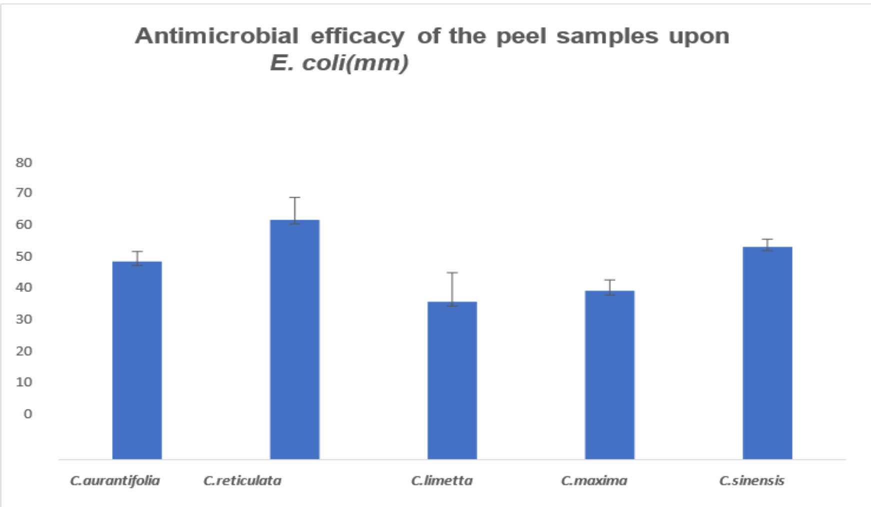

Fig. 4. Diagrammatic representation of the antimicrobial activity of the peel samples on *E. coli* bacteria

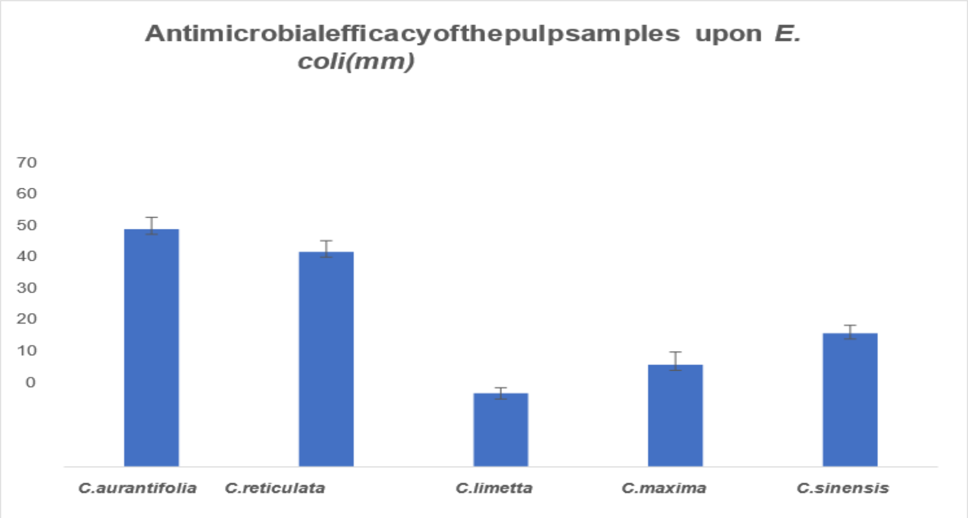

Fig. 5. Diagrammatic representation of the antimicrobial activity of the pulp samples on *E. coli* bacteria

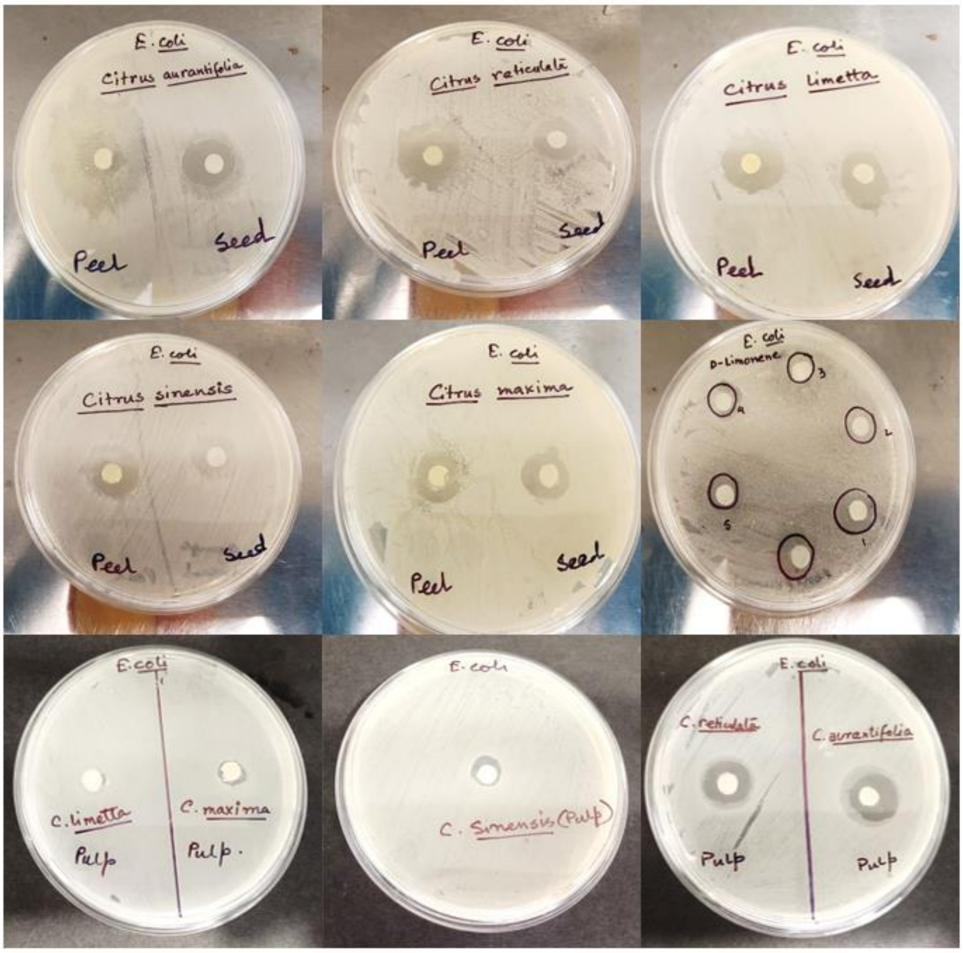

Fig. 6. Pictorial representation of the antimicrobial activity [zone of inhibition] of the seed peel and pulp samples on *E. coli* bacteria

Table 1. Inhibitory zones (mm) of three different parts of citrus fruits of five different species

Part	*Samples*	Zone of Inhibition(mm)
Seed	*C. aurantifolia*	58.33±0.471
	C. reticulata	47.66±3.09
	C. limetta	31±4.89
	C. maxima	57±3.55
	C. sinensis	45±3.45
Peel	*C. aurantifolia*	54±2.82
	C. reticulata	65.33±6.16
	C. limetta	43±8.06
	C. maxima	46±3.09
	C. sinensis	58±2.16
Pulp	*C. aurantifolia*	55.33±2.94
	C. reticulata	50±2.82
	C. limetta	17±1.63
	C. maxima	23.66±3.29
	C. sinensis	31±2.16

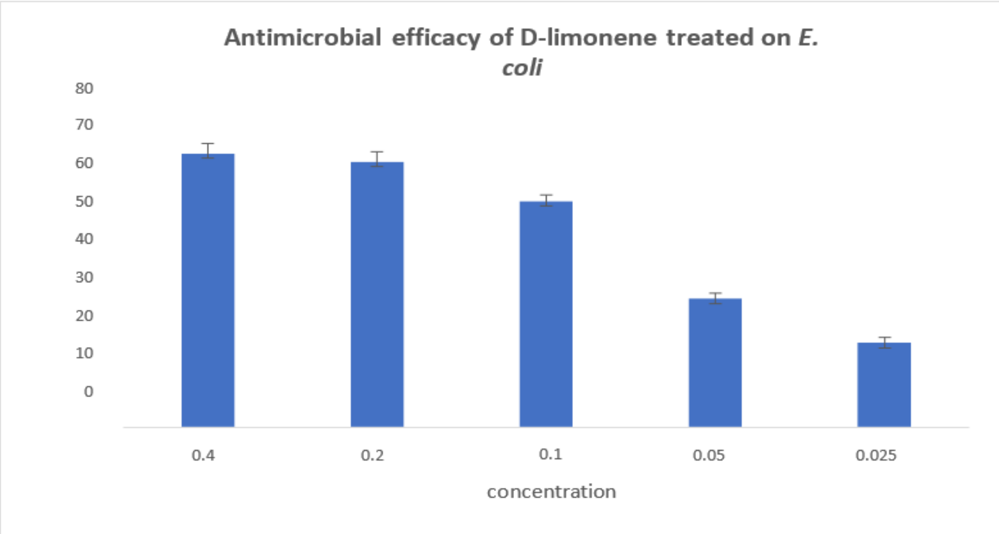

Fig 7. Different concentrations of D limonene have shown different inhibitory zones on *E.coli*

Fig. 8. Pictorial representation of the anti microbial activity of the bioactive compounds D-Limonene

Table 2. Range of concentration of d-limonene treatment along with their Inhibitory zones (mm)

Treatment	Inhibitory zones (mm)
0.4	65±2.44
0.2	63±2.5
0.1	53.66±1.6
0.05	30.5±1.5
0.025	20±1.5

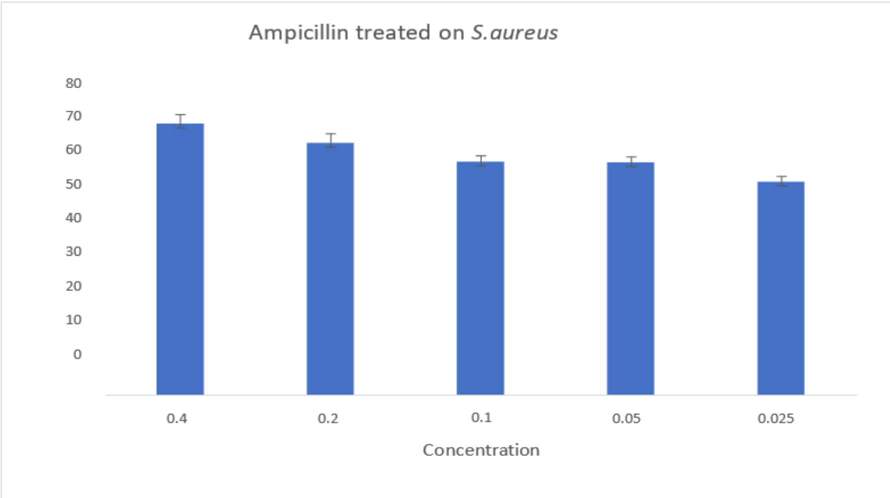

Fig. 9. Diagrammatic representation of the antimicrobial activity of Ampicillin on *S. aureus*bacteria

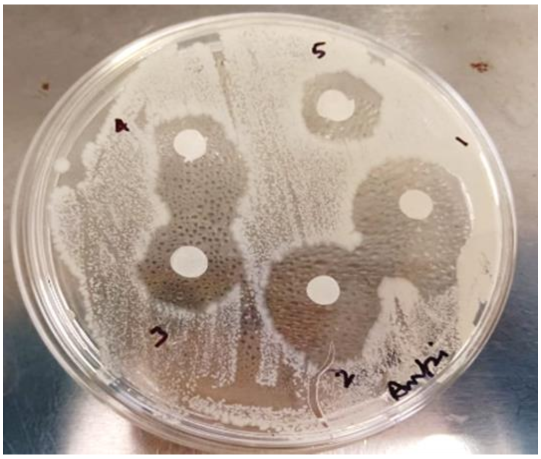

Fig. 10. Pictorial representation of the anti microbial activity of Ampicillin on *S. aureus* bacteria

3.2 Antimicrobial Efficacy of Samples against Gram-Positive Bacteria Staphylococcus *aureus*

Different concentrations of Ampicillin have shown different inhibitory zones on *S. aureus.* Every time 40 µl of the sample was used for the treatment 0.4 mg 70 ± 2.44 mm, 0.2 mg - 65 ± 2.5 mm, 0.1 – 60.2 ±1.6 mm, 0.05 mg - 60 ±1.5 mm, 0.025mg – 55±1.5 mm [Figs. 9 and 10].

The result shows *C. aurantiifolia* and *C.maxima* have the highest antimicrobial activity among other samples. Sd measured (n=3) for each of the samples Pictorial representations show the zones in which the samples have inhibited bacterial growth. Seed extracts of *C. aurantiifolia* (58.33 ±0.4 mm) and *C. maxima* (57 ±3.5 mm) showed good antibacterial efficacy.

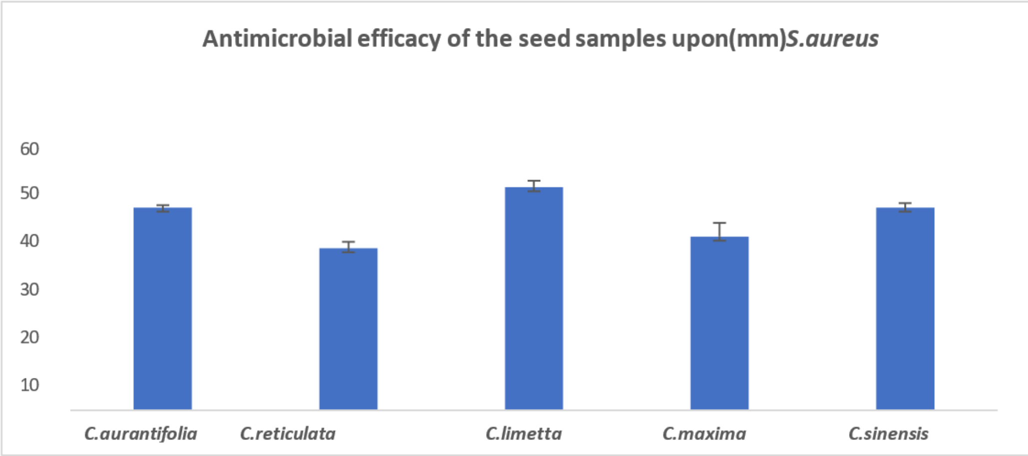

Fig. 11. Diagrammatic representation of the antimicrobial activity of the seed samples on *E. coli* bacteria

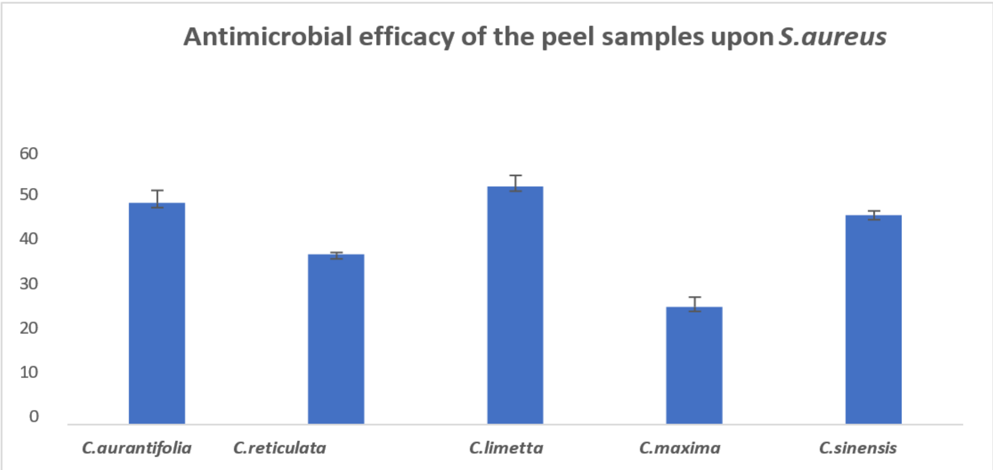

Fig. 12. Diagrammatic representation of the antimicrobial activity of the peel samples on *E. coli* bacteria

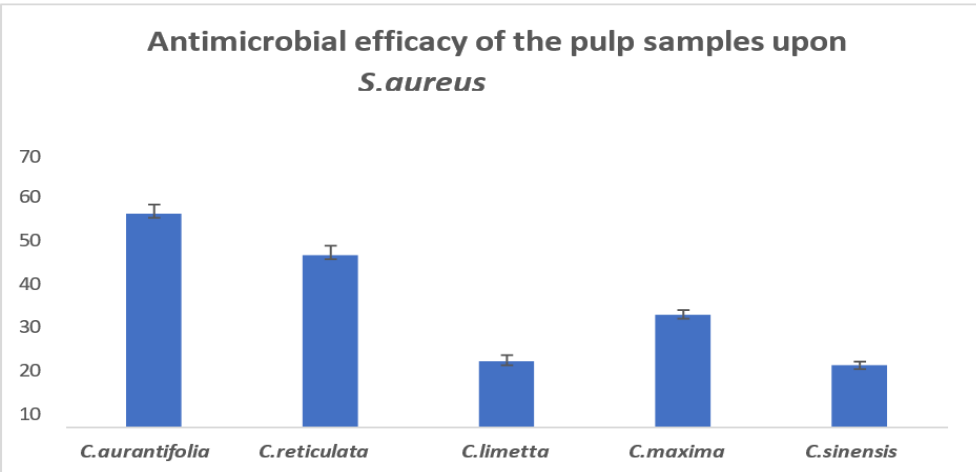

Fig. 13. Diagrammatic representation of the antimicrobial activity of the pulp samples on *E. coli* bacteria

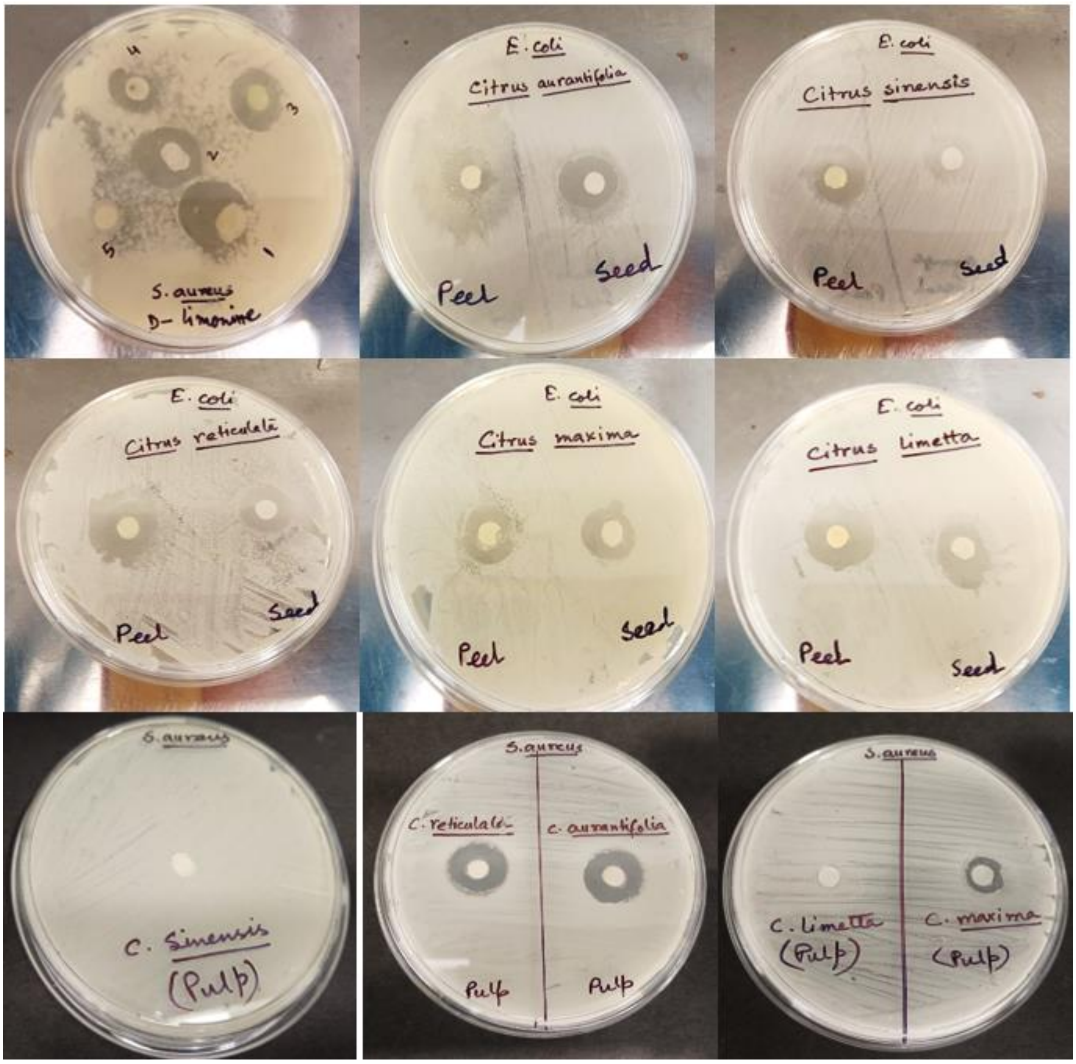

Fig. 14. Zone of inhibition of seeds, peels, and pulps of all five citrus species along with d-limonene against gram-positive bacteria Staphylococcus *aureus*

Table 3. Inhibitory zones (mm) of three different parts of citrus fruits of five different species

Part	Samples	Zone of Inhibition(mm)
Seed	*C. aurantifolia*	46.33±0.47
	C. reticulata	37±1.41
	C. limetta	51±1.41
	C. maxima	39.66±3.09
	C. sinensis	46.33±0.94
Peel	*C. aurantifolia*	49±2.82
	C. reticulata	37.66±0.4
	C. limetta	52.66±2.49
	C. maxima	26±2.16
	C. sinensis	46.33±0.943
Pulp	*C. aurantifolia*	55±2.44
	C. reticulata	44.33±2.49
	C. limetta	17±1.63
	C. maxima	29±1.24
	C. sinensis	16±0.94

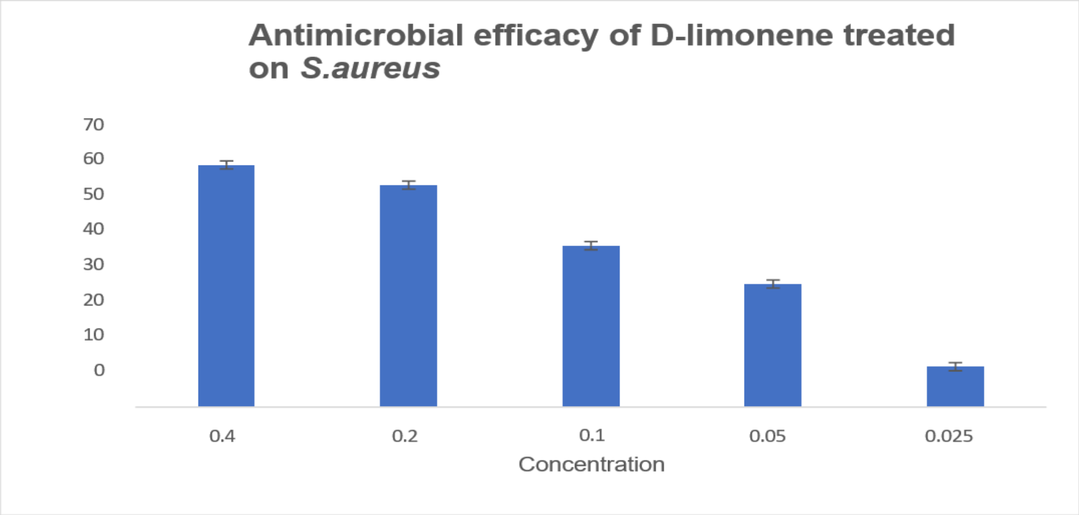

Fig. 15. Diagrammatic representation of the anti microbial activity of the bioactive compound D- Limonene on *S. aureus* bacteria

Fig. 16. A pictorial representation of the anti microbial activity of the bioactive compound D-Limonene on *S. aureus* bacteria

Table 4. Range of concentration of d-limonene treatment along with their Inhibitory zones (mm)

Treatment	Inhibitory zones (mm)
0.4	60±2.44
0.2	55±2.5
0.1	40±1.6
0.05	30.5±1.5
0.025	10±1.5

The seeds of citrus species *Citrus limetta* and *Citrus sinensis* both hada high zone of inhibition of 51±1.41mm and 46.33±0.94mm respectively against Staphylococcus aureus followed by *Citrus aurantifolia* with a zone of 46.33±0.47mm. In the case of peels, *Citrus limetta* gave the highestzone ofinhibition of 52.66±2.49mm followed by *Citrus aurantifolia* with a zone of 49±2.82mm. In the case of the pulp portion of the fruit,*Citrus aurantifolia* gave the highest zone of inhibition of 55±2.44mm followed by *Citrus reticulata* with an inhibition zone of 44.33±2.49mm. [Fig. 14].

Different concentrations of D limonene have shown different inhibitory zones on *S. aureus*. Every time 40 µl of the sample has been used for the treatment 0.4 mg 60 ± 2.44 mm, 0.2 mg - 55± 2.5 mm, 0.1 – 40 ±1.6 mm, 0.05 mg - 30.5 ±1.5 mm, 0.025mg – 10 ±1.5 mm [Figs. 15, 16].

4. DISCUSSION

The significant antimicrobial activities observed after treating three different parts of lemons can be attributed to the bioactive compounds present, particularly limonene. Limonene is a monocyclic monoterpene with strong antioxidant qualities [20] that can scavenge reactive oxygen species and react with bacterial proteins due to its basic characteristics. The natural compounds present in our samples demonstrate therapeutic behavior and can exhibit antimicrobial activities.Ampicillin is a common β-lactam antibiotic in clinical settings and used as a control in microbiological research. It has been validated for quantitative analysis in injectable solutions using turbidimetric tests and Staphylococcus aureus as the test microorganism [21]. *Escherichia coli* is a versatile bacterium found in various environments, including the gastrointestinal tracts of warm-blooded animals [22,23]. While E. coli has traditionally been used as an indicator for fecal contamination, recent research has shown that certain strains of the bacteria can survive and thrive outside the intestines, calling into question its accuracy as an indicator [22]. *E. coli* exhibits genetic diversity and subspecies structure, with different groups inhabiting various ecological niches and following different life history strategies [24]. Some strains have the potential to cause enteritis and other illnesses in humans and animals [25,24]. Environmental factors affect the genetic makeup and long-term survival of E. coli populations [22,24]. Understanding the life history and ecology of E. coli can improve its usefulness as a model organism and has implications for assessing water quality and controlling the spread of disease [23,24] Seeds of *C.aurantifolia (*58.33±0.471) and *C. maxima* (57 ±3.5 mm) showed good antibacterial efficacy [Fig. 6] peel extracts of *C. reticulata* (58.33 ±0.4 mm) and *C. sinensis* (57 ±3.5 mm) showed good antibacterial efficacy. Pulp extracts of *C. aurantiifolia* (55.33 ±2.94 mm) *and C. reticulata* (50±2.6 mm)showed good antibacterial efficacy [Fig. 6] Every time 40 µl of the limonene sample has been used for the treatment 0.4 mg 65± 2.44 mm, 0.2 mg - 63 ± 2.5 mm, 0.1 – 53.66 ±1.6 mm, 0.05 mg - 30.5 ±1.5 mm, 0.025mg – 20 ±1.5 mm [Figs. 7, 8] and the *S.aureus baceteria* the result shows Seed extracts of *C. aurantiifolia* (58.33 ±0.4 mm) and *C. maxima* (57 ±3.5 mm) showed good antibacterial efficacy. Staphylococcus aureus, a common bacterium, is responsible for many infections acquired in the community and in hospitals [26,27]. It can cause a wide range of diseases, from minor skin infections to more serious conditions such as endocarditis, sepsis, and toxic shock syndrome, as it possesses multiple characteristics that allow it to evade the body's defenses [26,27,28]. S. aureus can colonize human skin and mucous membranes, with about 30% of people being chronic carriers [27]. The bacterium's ability to form biofilms on medical devices facilitates healthcare-associated infections [26]. Methicillin-resistant S. aureus (MRSA) is becoming increasingly common in hospitals and communities due to its resistance to several antibiotics, posing a serious risk to public health [26,29,28]. S. aureus infections have a significant financial cost and impact both human and animal health [28]. In the case of peel extracts, *Citrus limetta* gave the highest zone of inhibition of 52.66±2.49mm followed by *Citrus aurantifolia* with a zone of 49±2.82mm. In the case of the pulp portion of the fruit,*Citrus aurantifolia* gave the highest zone of inhibition of 55±2.44mm followed by *Citrus reticulata* with an inhibition zone of 44.33±2.49mm. [Fig. 14] Different concentrations of D limonene have shown different inhibitory zones on *S. Aureus*. Every time 40 µl of the sample has been used for the treatment 0.4 mg 60 ± 2.44 mm, 0.2 mg - 55± 2.5 mm, 0.1 – 40 ±1.6 mm, 0.05 mg - 30.5 ±1.5 mm, 0.025mg – 10 ±1.5 mm [Figs. 15, 16].

5. CONCLUSION

In our research, we discovered that various parts of the citrus fruit exhibit a wide range of antimicrobial effects against both gram-positive and gram-negative bacteria. Our findings indicate that *citrus reticulata* peels demonstrated the most significant zone of inhibition at 65.33±6.16mm against E. coli, while the pulps of *citrus aurantifolia* displayed the highest zone of inhibition at 55±2.44mm against S. aureus. Furthermore, d-limonene, an essential bioactive compound present in different citrus species, exhibited the highest zone of inhibition.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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