EXPLORING THE ANTIMICROBIAL PROPERTIES OF LEMON: A COMPARATIVE ANALYSIS OF PEEL, SEED, AND PULP

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.

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.

Result

The results show that in the case of E. coli inhibition (water extractions), the peels of C. reticulata, the seeds of C. aurantifolia, and the pulp of C. aurantifolia perform well compared to other samples. In the case of S. aureus inhibition, the peels of C. limetta, the seeds of C. sinensis, and the pulp of C. aurantifolia 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

CitrusfruitbelongstotheRutaceaefamily.Theyarerichincitricacidandascorbic acid,strengtheningourimmunesystem.Citrusfruitsareoneofthemostwidelygrownfruitsandareanimportantsourceofphysiologicallyactivesubstancesandphytochemicals (Suri, Singh, and Nema 2022). Theycan potentiallybeemployedasanaturalmedicationsourceoramajorcomponentoffunctionalfoodsincetheyareanexcellentsourceofbioactivecompoundsandarehighinascorbicand citricacid,whichboostsourimmunesystems.Thesecompoundsaremostlyrecognizedintheirednibleparts. Ontheotherhand,limonoids—typicalcitrusfruittriterpenoidswithanintenselybittertasteandpotentiallyanticarcinogenicandchemo-preventivepropertiescanbeextractedfromseeds.(Sengupta et al. 2023).

Citrusfruitsincreasewastegenerationwhenconsumedraworjuiced.Conventionalrubbishdisposaltechniquescontaminatelandandwaterways,potentiallyharmingaquaticenvironments.Consequently,tolessen thequantityofwastethatbuildsup

Intheenvironment,itisessentialtocreatecreativeprocessesandeconomical,ecologicallyfriendlywastemanagementtechniques(Seid et al. 2023).

Bacterial infections worldwidearealeadingcauseofillnesses,physicalimpairments,anddeaths.Medicinalplantsarethoughttoofferasaferandmoreaffordablealternativefortreatingbacterialinfectionsbecausetheycontainadiverse arrayofphytochemicals. Naturalmedicinesmadefrommedicinalplantshaveantibacterialpropertiesthatcanbeusedtotreatviral,fungus,andbacterialillnesses.Microorganismsarebecomingmoreresistanttoantibiotics,despitethepharmaceuticalindustryhavingdevelopedseveralnewonesduringthepastthreedecades.(Gupta and Sharma 2022)Manyyearsofantibacterialstudyhavebeenconductedon citrus peels, pulp, and seeds, and it has been demonstrated that seed wastes consist of a substantial number of essential oilsand polyphenols with antimicrobialactivity.Bioactivechemicals(Roquia et al. 2022) with potential medicinalapplications can be found in abundance in citrustrash.Numerousadvantageouscharacteristicsofthesecompounds have been discovered, such as their anti-aging, anti-mutagenic, anti-carcinogenic, and anti-allergeniceffects.Oneparticularlyinterestingsourceofnaturalchemicalswithpotentialfortherapeuticuseis Citrussinensis.Newmedicationsmaythereforebedevelopedasaresultofmoreresearchintotheextractionandapplicationofthesesubstancesfromcitrustrash.(Baker, Ibrahim, and Salama 2021)

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.

LITERATURE STUDY

(Mohammed and Ayoub 2016)identifiedthepresenceofalkaloids,saponins,andothercompoundsintheseedsthatcontributetotheiranti microbialactivity(Valarmathy et al. 2010)furtherhighlightedtheantimicrobial effects of Citrus limetta, attributing them to the presence of limonene and othercompounds.

(Al-Aamri et al. 2018)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. Incontrasttoampicillin,thepositivecontrol,itsantibacterialactivityissignificantlylower.

(Shakya et al. 2019)showed citrus reticulata extracts have antibacterial efficacy against specificstrains of bacteria with a maximal zone of inhibition against Bacillus sp. citrus fruit peel extract wasfoundtobemoreefficientthanjuiceextractagainstgram-positivebacteriawithahighinhibitionzoneagainst Klebsiella pneumoniae the extract of juice was shown to be more efficient than its peelextractamonggram-negativebacteriaalthough10DMSO showednozoneofinhibitionCitruslimetta. antibacterial effectiveness against particular bacterial strains compared to its juice extract the peelextractshownagreaterabilitytoinhibitgrampositivebacteriawithamaximalinhibitionzoneagainstBacillus sp. with the exception of Klebsiella pneumoniae on which the peel extracts was moreeffective with a inhibition zone DMSO did not exhibit any zone of inhibition among the gram negativebacteriathejuiceextractofc2wasfoundtobemoreeffectivethanitspeelextractagainstthefourotherbact eria chosen with maximum zone of inhibition against E coli Citrus maxima exhibit antibacterialactionagainstspecificstrainsofmicroorganismstheresultsindicatethatpeelextractwasmoreefficie ntagainst Bacillus sp among gram positive bacteria whereas its juice extract was more effective against Saureus and S aureus with a maximum zone of inhibition against E coli the juice extract of wasshown to be more efficient against gram negative bacteria than its peel extract although 10 dmsoshowednoinhibitionzone.

Accordingto(Garba et al. 2016)Caurantifoliaisrichinphytochemicalcomponentssuchasflavonoids alkaloidstanninsandphenolsallofwhichhavebeenshowntohaveantibacterialqualities.

(Sahlan et al. 2018) 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.

(Al–Âni, Tawfik, and Shehab 2011) stated that it is most likely that this 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

(Lahlou 2004)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.

(Arora and Kaur 2013) found pulp has more antioxidant activity and antimicrobial activity as compare with the orange peel.

(Han, Chen, and Sun 2021)found 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.

2. MATERIAL AND METHODS

2.1 MATERIAL

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. (Sulaiman et al. 2011). 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(Mediani et al. 2013) .D-limonene source from Sigma-Aldrich

2.1.2 Microorganisms

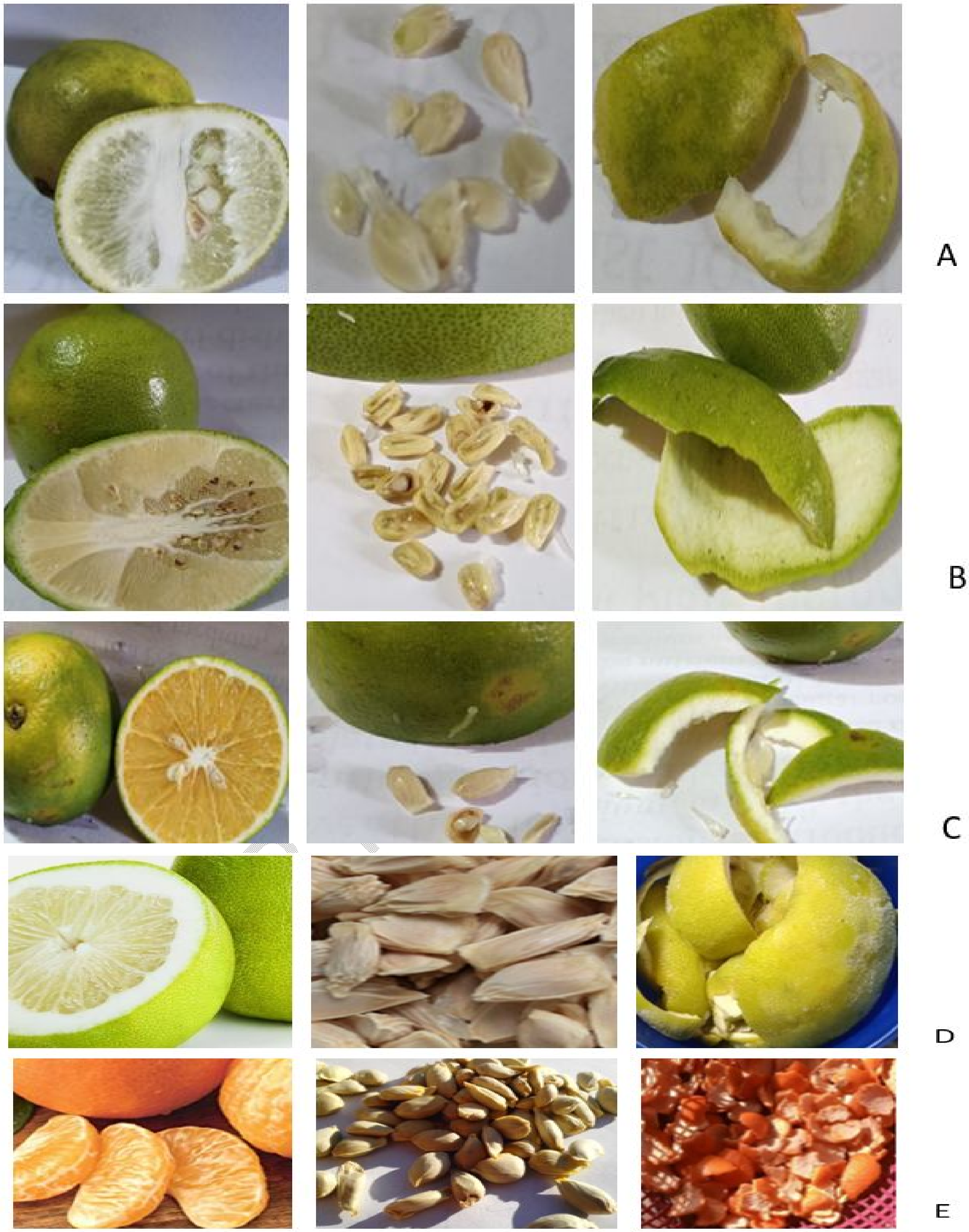
Staphylococcus aureus and Escherichia coli bacterial strains were obtained from the Department of Biotechnology, Techno India University, West Bengal, Salt Lake, Kolkata-700091. The bacteria strains were grown in Himedia M002-100G Nutrient broth at 37^0 Celsius or 98.6 Fahrenheit in a shaker incubator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

1 **2** **3**



Pic 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*

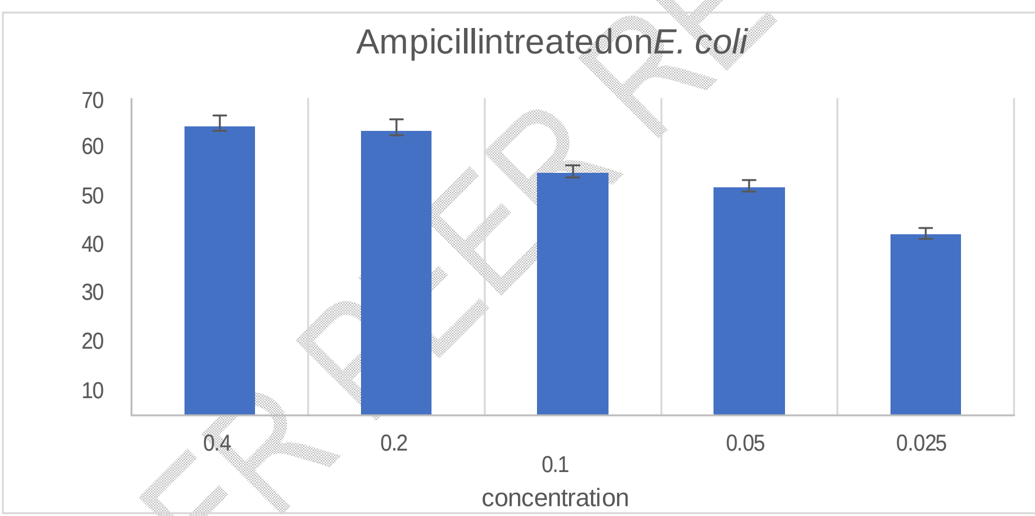


Fig 1: Diagrammatic representation of the antimicrobial activity of Ampicillin on *E. coli* bacteria

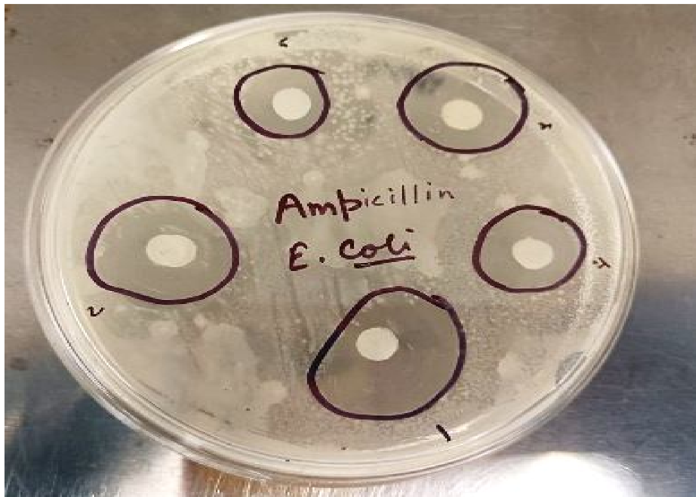


Fig 2 : a pictorial representation of the inhibitory zones of ampicillin

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]

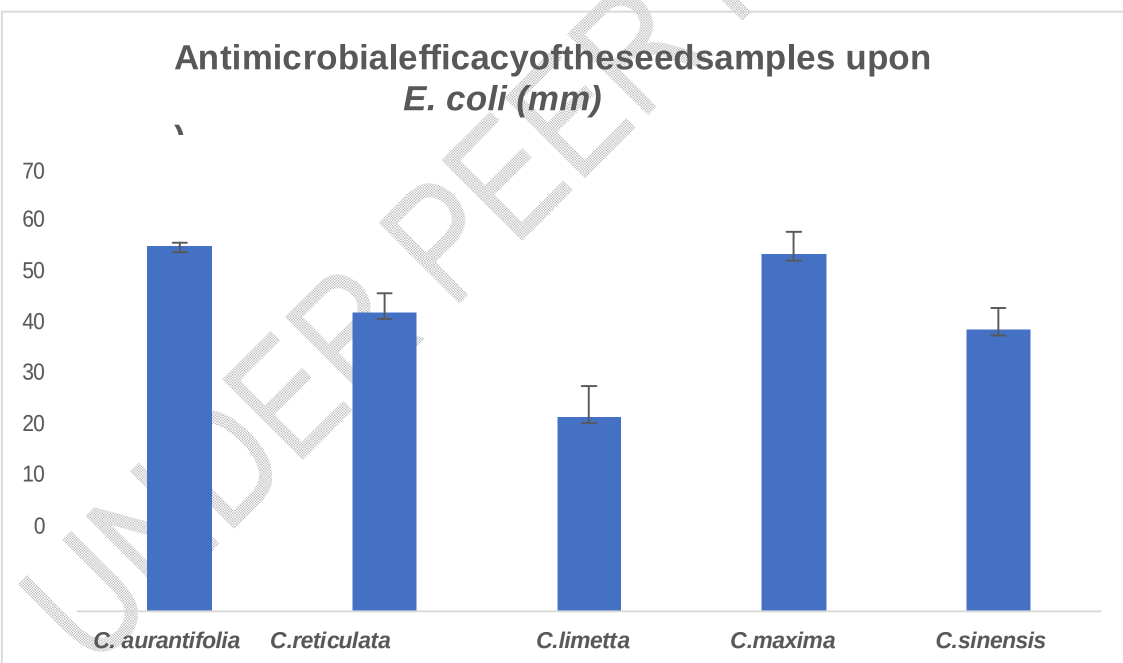


Fig. 3: Diagrammatic representation of the antimicrobial activity of the seed samples on *E. coli* bacteria.

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]**

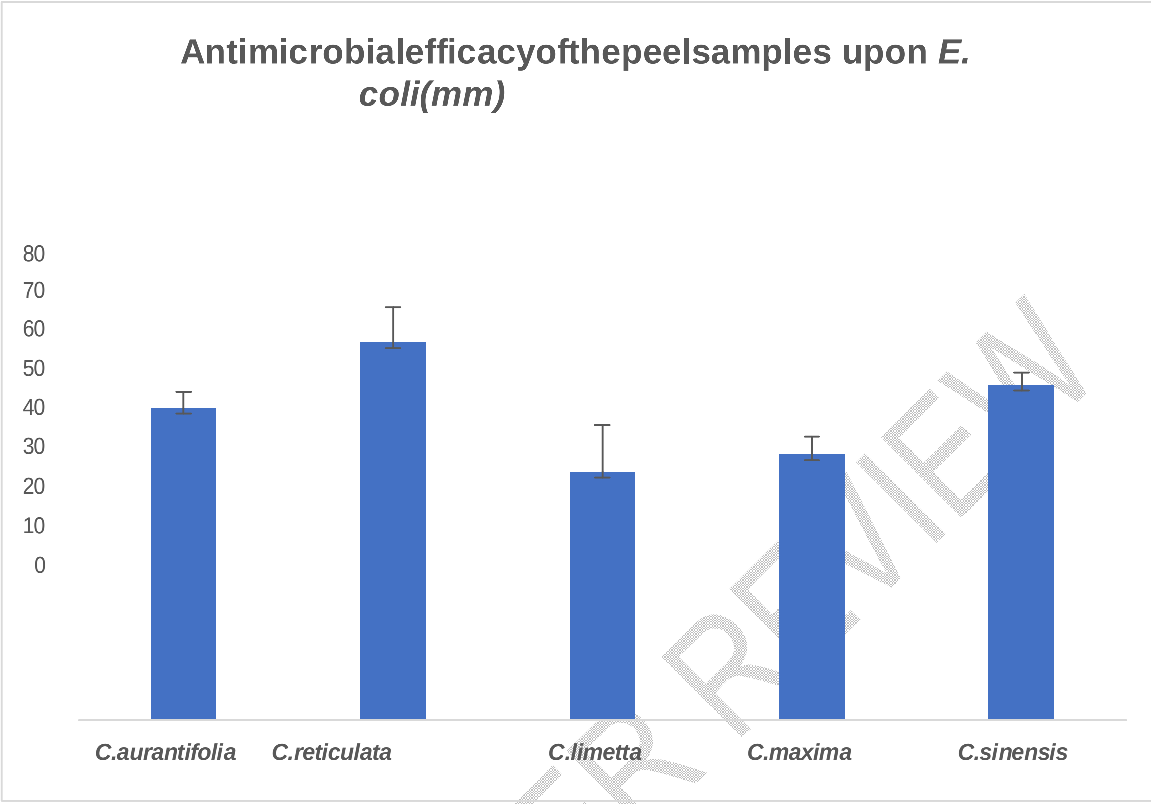


Fig. 4: Diagrammatic representation of the antimicrobial activity of the peel samples on *E. coli* bacteria

C. reticulata and*C.aurantiifolia*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. Seed extracts of *C. aurantiifolia* (58.33 ±0.4 mm) and *C. maxima* (57 ±3.5 mm)showed good antibacterial efficacy

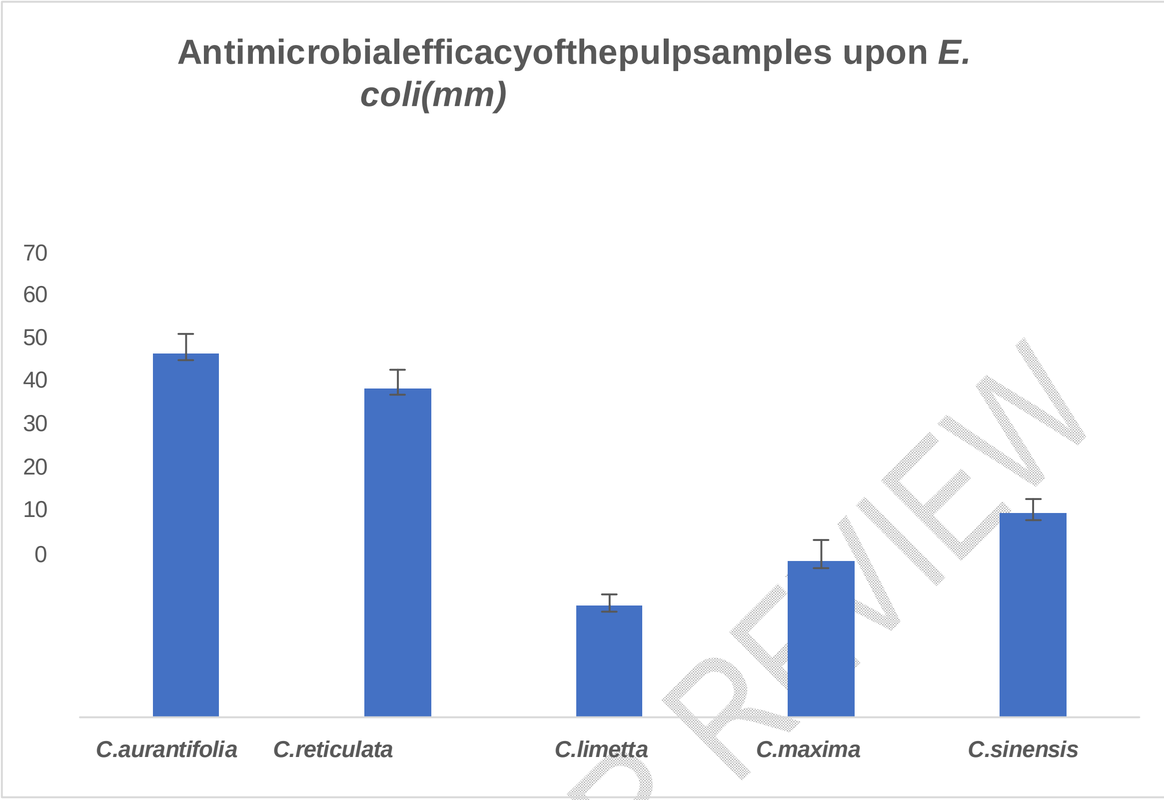


Fig. 5: Diagrammatic representation of the antimicrobial activity of the pulp samples on *E. coli* bacteria

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]**

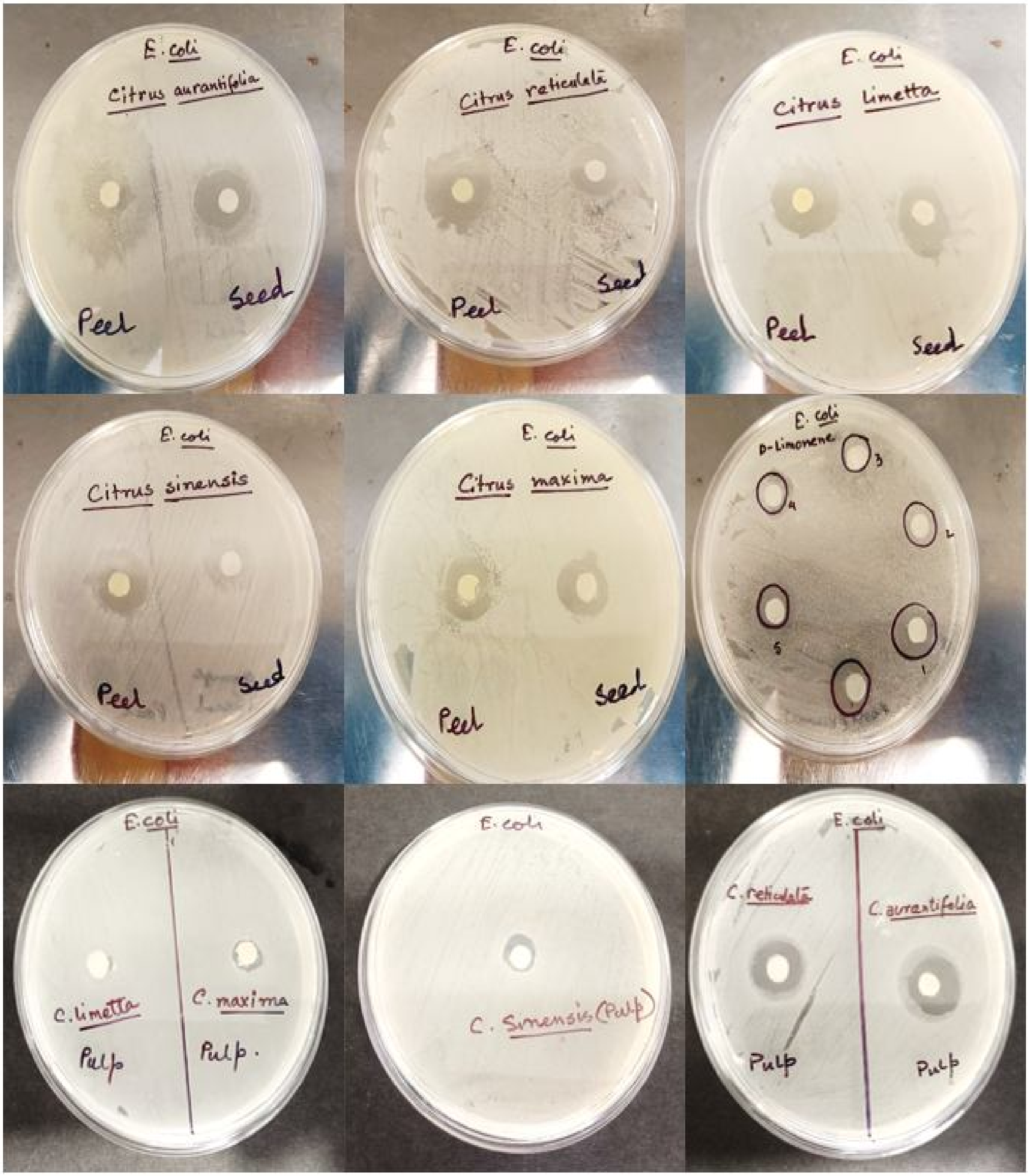


Fig. 6: Pictorial representation of the antimicrobial activity [zone of inhibition] of the seed peel and pulp samples on *E. coli* bacteria.

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

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

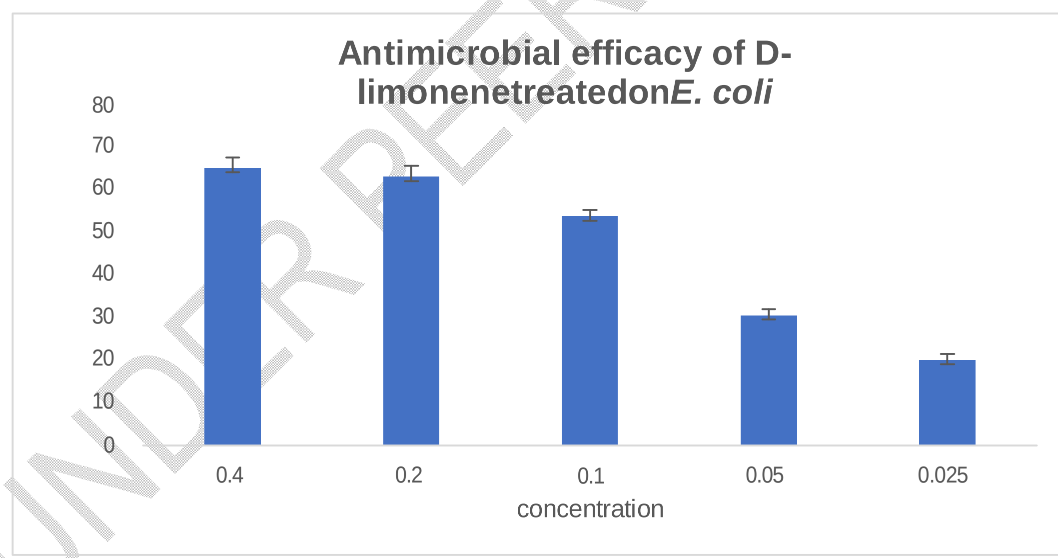


Fig 7: Different concentrations of D limonene have shown different inhibitory zones on *E.coli*.

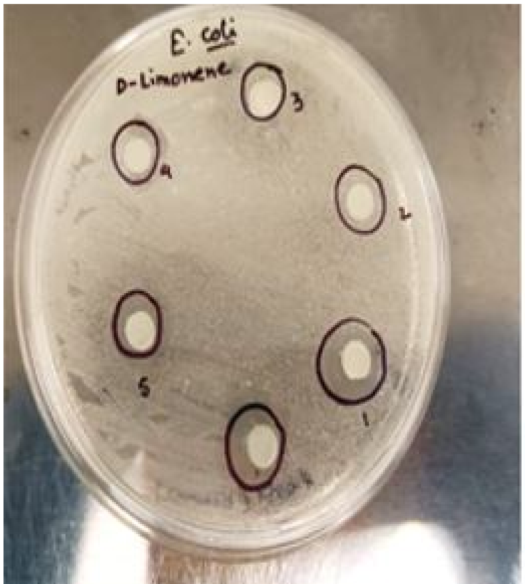


Fig 8: Pictorial representationoftheantimicrobialactivityofthe bioactive compounds D-Limonene

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

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

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 **[fig 7, 8]**

3.2 Antimicrobial efficacy of samples against gram-positive bacteria Staphylococcus *aureus*

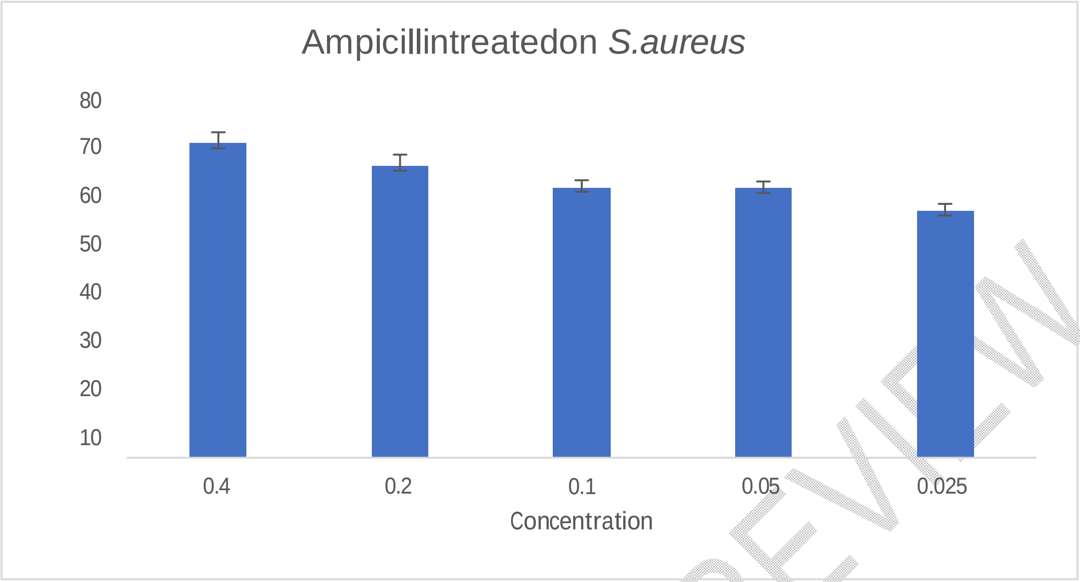


Fig. 9: Diagrammatic representation of the antimicrobial activity of Ampicillin on *S.aureus*bacteria

Fig. 10:PictorialrepresentationoftheantimicrobialactivityofAmpicillin on *S. aureus* bacteria

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 [fig 9 and 10]

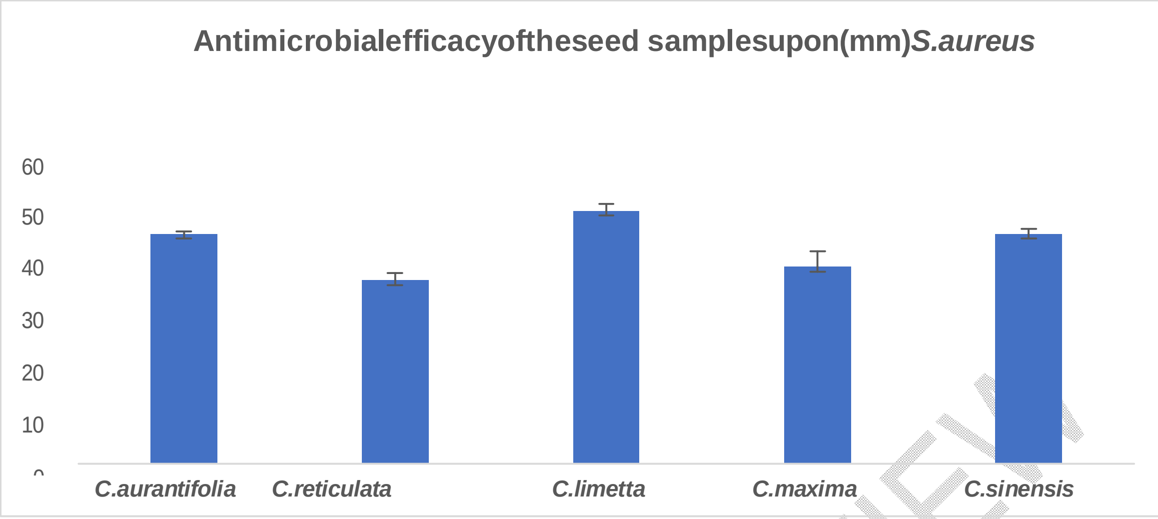


Fig 11 : Diagrammatic representation of the antimicrobial activity of the seed samples on *E. coli* bacteria.

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

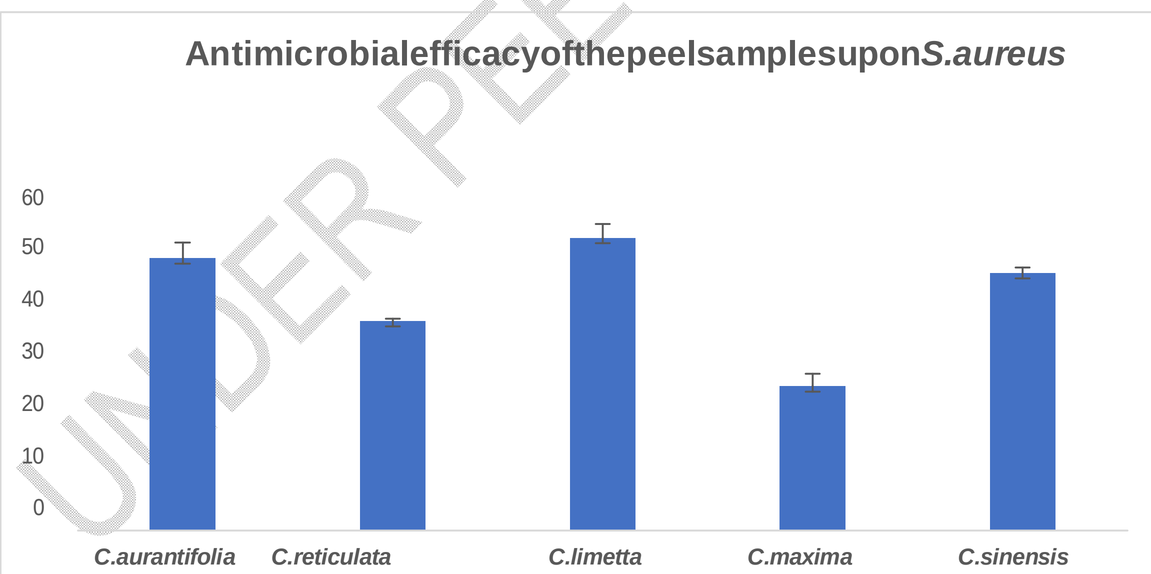


Fig 12: Diagrammatic representation of the antimicrobial activity of the peel samples on *E. coli* bacteria.

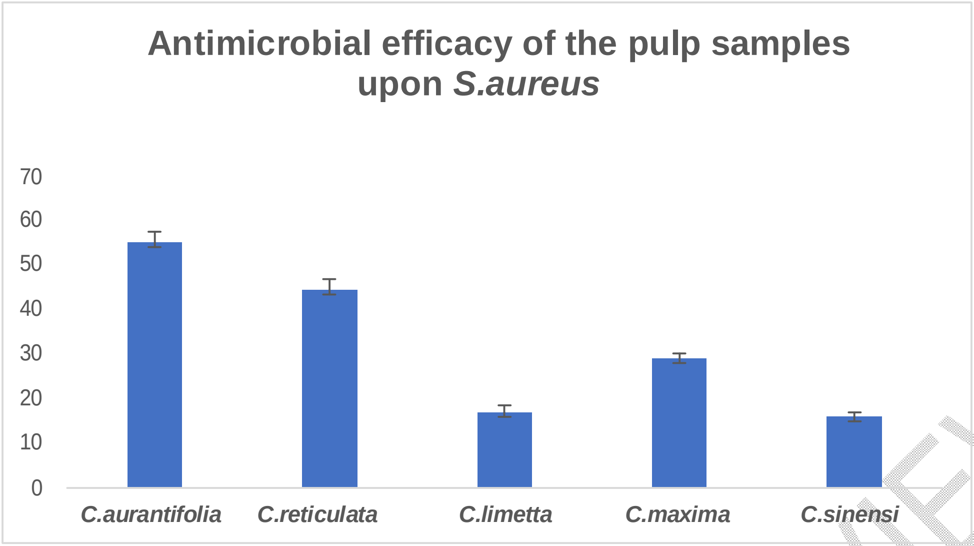


Fig 13: Diagrammatic representation of the antimicrobial activity of the pulp samples on *E. coli* bacteria.

The seeds of citrus species Citrus *limetta* and Citrus *sinensis* bothgave 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 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]

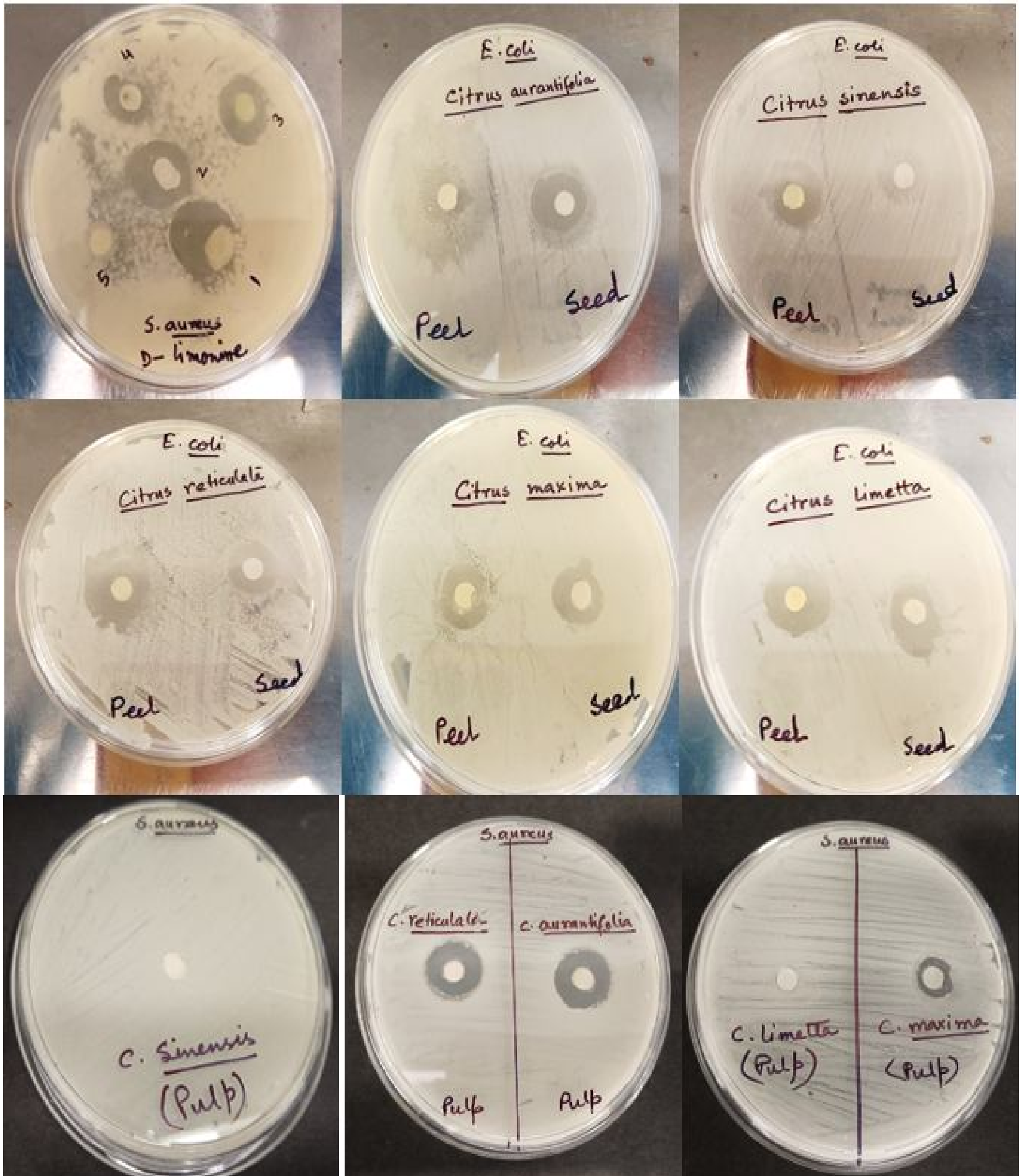


Fig. 14. Zone of inhibition of seeds, peels, and pulps of all five citrus species along with d-limonene against gram-positive bacteria Staphylococcus *aureus*

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

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

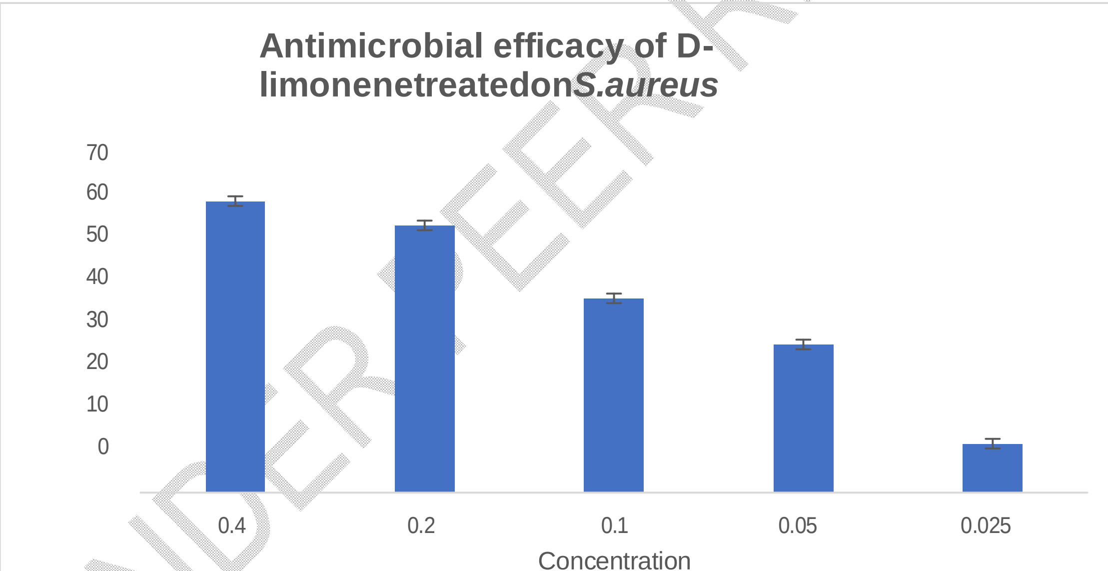


Fig 15: Diagrammaticrepresentationoftheantimicrobialactivityofthe bioactive compound D-Limonene on *S. aureus* bacteria

Fig 16: A pictorial representationoftheantimicrobialactivityofthe bioactive compound D-Limonene on *S. aureus* bacteria

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

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

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 **[fig 15 16]**

4. 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.

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