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ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL AND METHANOLIC EXTRACTS OF SWEET LIME (*CITRUS LEMITTA*) FRUIT

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ABSTRACT

Sweet lime (*Citrus lemitta*) fruit was collected from the Lahore Punjab Pakistan vegetable market. Essential oil from their peel, methanolic extracts from peel, pulp, and seed were extracted. The antimicrobial activities of essential oil and methanolic extract were evaluated using the disc diffusion method. Essential oil and methanolic extracts of sweet lime peel, pulp, and seed showed percentage inhibition against *Escherichia coli* were 17mm, 9mm, 10mm, and 5mm, *Salmonella Typhi* was 20mm, 8mm, 12mm, and 5mm and *Staphylococcus aureus* were 12mm, 11mm, 12mm, and 8mm respectively by using *Streptomycin* as a standard. Antifungal activity of essential oil and methanolic extracts of sweet lime seed, pulp, and peel against *Aspergellius niger* was 40mm, 38mm, 20mm, and 35mm, *Aspergillus alternata* were 22mm, 18mm, 18mm, and 23mm and *Aspergillus terreus* were 17mm, 11mm, 20mm, and 20mm respectively and compare these results with *1% fluconazole* as a standard. The citrus peel oil showed strong antimicrobial activity.

KEYWORDS: Sweet lime; Essential oil; Methanolic extract; Disc diffusion method; Antibacterial activity; Antifungal activity.

INTRODUCTION

Medicinal plants contain chemical components with therapeutic value and, therefore have been employed as treatments for human diseases (Nostro *et al*., 2000). Ten species of the genus Citrus are found in Pakistan (Omer & Qaiser, 1993). Sweet lime is one of them. In Punjab, Pakistan it is known as "Mitha". It is sweet, flavorful, full of juice and vitamin C. About 4% of the world's citrus production comes from Pakistan, and its export contribution is merely 0.8%. (Tahir, 2014). According to the traditional indigenous medical system, sweet lime juice has therapeutic benefits in treating fever, malaria, and jaundice (Cowan, 1999). Any substance used to treat or prevent infections that directly affect microorganisms is referred to as an antimicrobial. Antibiotics include antifungals, antivirals, antibacterials, and antiprotozoals. To preserve food quality and increase the shelf life of foods and beverages, naturally occurring antimicrobial substances could be used as food preservatives. A wide variety of plants produce secondary metabolites with antibacterial qualities (Ushimaru *et al*., 2007).

The essential oil extracted from citrus fruit contain phenolic components in higher quantity which are known to be the bioactive elements responsible for their antibacterial properties (Rodrigues *et al*., 2000). Ascorbic acid, or vitamin C, and secondary metabolites such phenolic acid, coumarins, carotenoids, flavonoids, alkaloids, and limonoids are most abundant in citrus fruits (Banerjee & Pal, 2021).

The research showed that essential oil of sweet lime exhibited maximum zone of inhibition against Bacillus cereus (28 mm) and Bacillus subtilis (26 mm) followed by Staphylococcus aureus (21 mm), whereas the minimum zone of inhibition was shown by Fusarium oxysporum (11 mm) after 48 h of incubation at their respective temperature (37°C for bacteria and 25°C for fungi) (Javed *et al*., 2013). Another study showed that sweet lime fruit-peel combo vinegar showed the strongest inhibition for the growth of E. coli and Salmonella typhi, while Sweet lime peel vinegar showed the highest zone of inhibition for the growth of Klebsiella species (Priyadarshini *et al*., 2014).

The current study was designed to thoroughly evaluate the waste materials from *citrus lemitta*, which include peels, pulp, and seeds. Essential oil was extracted from the peels and

after that methanolic extract of waste materials was prepared to evaluate their antimicrobial activities.

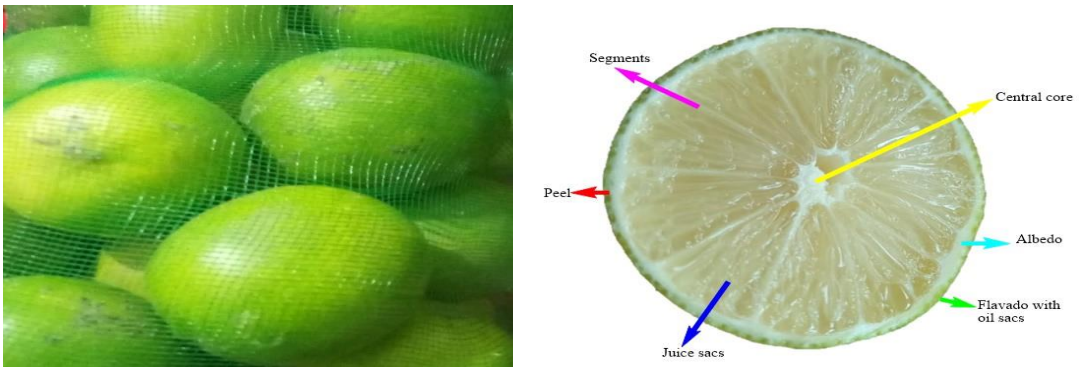

Figure 1: Sweet lime fruit.

MATERIAL AND METHODS

The experiment was designed to analyze the antimicrobial activity of essential oil and methanolic extracts of sweet lime fruit.

Collection of Material

Sweet lime fruit was collected from the local market, washed, and peeled. The essential oil was extracted from its peels through the hydrodistillation method and the methanolic extract was obtained from its peel, seed, and pulp.

Chemicals

Methanol, nutrient agar, potato dextrose agar(PDA), bacterial and fungal grown cultures were used for the research.

Preparation of Methanolic Extract

Sweet lime peel, pulp, and seed were dried and ground into powder. Methanolic extract of powdered peel, pulp, and seed was prepared through maceration with methanol. Solid to liquid ratio was 1:5 and it as macerated for 15 days with frequent shaking. After that mixture was filtered using filter paper to separate the solid residue from the liquid extract. The liquid extract was concentrated by eliminating the solvent through a distillation method. Then the resulting concentrates are stored in airtight sample bottles at room temperature.

Figure 2: Methanolic extracts of peel, seed and pulp.

Essential Oil Extraction

Fresh peels of sweet lime fruit were cut into small pieces. Essential oil is extracted from these peels through hydro-distillation utilizing a Linkersson-type apparatus for 10 hours (Shahzad *et al*., 2009). Essential oil was then dried by utilizing anhydrous sodium sulfate. After solvent removal, a transparent-colored oil was obtained. This dried oil is carefully preserved in a sealed bottle at 3°C in the fridge.

Figure 3: Essential oil.

Determination of Antimicrobial Activity

The antibacterial and antifungal activities of methanolic extract of sweet lime seed, peel, pulp, and essential oil, were assessed utilizing the agar disc diffusion method, by following the process outlined by (Baydar *et al*., 2004). Various pathogens including *Salmonella Typhi, Escherichia coli, and Staphylococcus aureus,* as well as fungi such as *Aspergellius nigar, Aspergellius terreus, and Alternaria alternate,* were used in performing the antimicrobial activity. Standard culture media of PDA (potato dextrose agar) and NA (nutrient agar) were used at appropriate temperatures (25°C for fungi, and 37°C for bacteria). The growth media was prepared, autoclaved, and transferred hygienically to sterilized petri plates.

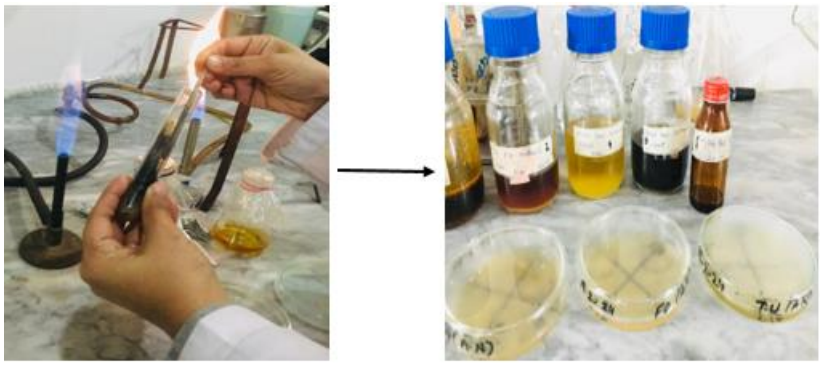

Figure 1: Determination of antimicrobial activity.

Microbial cultures from test tube slants were inoculated onto their respective media petri plates. Sterile, dried 6 mm paper discs impregnated with 20 µL of essential oil and sweet lime seed, peel, and pulp methanolic extracts. Discs, including a control, were kept on freshly inoculated microbial plates, with five discs on each plate. *Streptomycin* (20 µL/disc) served as the positive control for bacteria, and 1 1% *fluconazole* (20 µL/disc) for fungi. All experiments were conducted in triplicate. The Petri plates were subsequently incubated at their designated temperatures, and zones of inhibition against the tested microorganisms were examined in mm after 24 hours for bacteria and after 48 hours for fungi.

RESULTS AND DISCUSSION

Antimicrobial Activities of Sweet Lime

Both antibacterial and antifungal properties of sweet lime essential oil, along with methanolic extracts from sweet lime peel, seed, and pulp, were assessed for their efficacy against several pathogenic bacteria such as *Escherichia coli, Salmonella Typhi* and *Staphylococcus aureus,* as well as various fungi including *Aspergillus nigar, Aspergillus alternate* and *Aspergillus terreus.* The disk diffusion method was employed to measure the growth inhibition zone after the 24-hour incubation period.

Antibacterial Activities of Sweet Lime

The antibacterial potential of essential oil and methanolic extract of sweet lime peel, seed, and pulp determined against *Escherichia coli, Salmonella Typhi,* and *Staphylococcus aureus.* Significant zones of inhibition were observed against these bacteria. The subsequent figures illustrate the inhibition zones caused by essential oil and methanolic extracts from sweet lime pulp, peel, and seed against *Escherichia coli, Salmonella Typhi,* and *Staphylococcus aureus.*

In Figure 5, different numbers represented the samples employed for determining the antibacterial activities with zones of inhibition. Among these numbers, 5 showed a zone of inhibition of essential oil, 6 showed a methanolic extract of sweet lime pulp, 7 showed a methanolic extract of sweet lime seed, 8 showed a methanolic extract of sweet lime peel and the center showed the zone for standard which was Streptomycin.

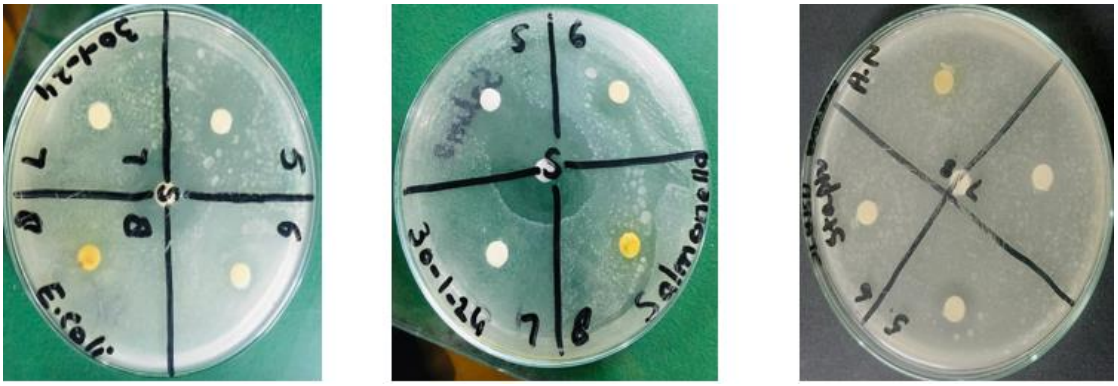

Figure 2: Determination of antibacterial activity.

Table 1: Samples showed zones of Inhibition (mm) against bacteria.

Tested Organisms	Standard (Streptomycin)	Essential Oil	Methanolic Extract of Sweet lime Peel	Methanolic Extract of Sweet lime Seed	Methanolic Extract of Sweet lime Pulp
Escherichia coli	22	17	9	6	10
Salmonella typhi	30	20	8	5	12
Staphylococcus aureus	20	12	11	8	12

The comparison of these results is alsoillustrated in the following graph.

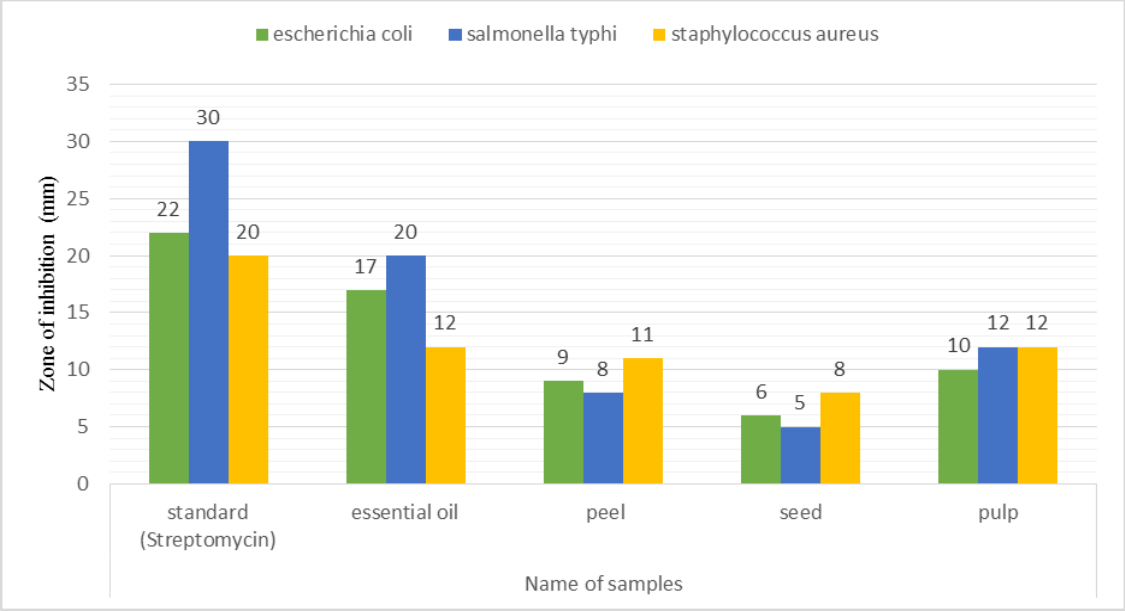

Figure 6: Graph of antibacterial activity of essential oil and methanolic extracts.

Antifungal Activities of Sweet lime

The essential oil and methanolic extracts of sweet lime seed, pulp, and peel tested against *Aspergillus niger*, *Aspergillus alternata*, and *Aspergillus terreus*. They showed %age inhibition against the tested fungi. The following figures showed the zones of inhibition of essential oil and methanolic extracts of pulp, peel, and seed against *Aspergillus niger*, *Aspergillus alternata*, and *Aspergillus terreus* fungi.

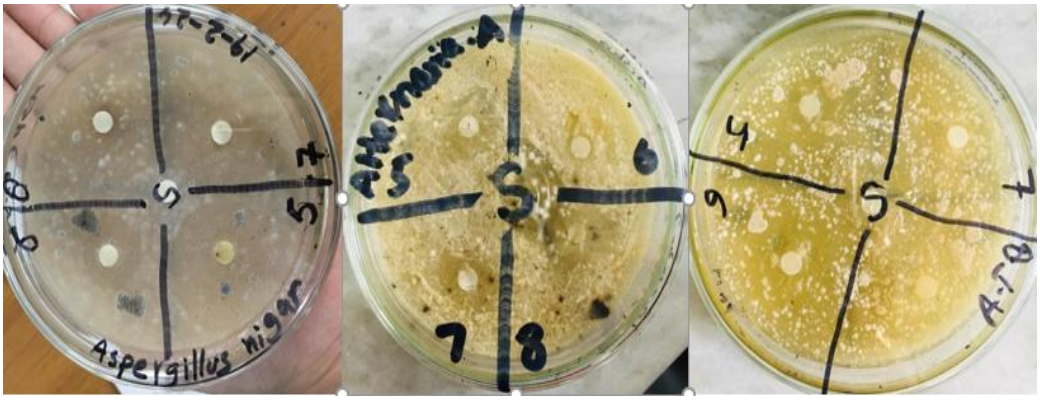

Figure 3: Determination of antifungal activity.

In Figure 7, different numbers represented the samples employed for conducting antifungal activities with zones of inhibition. Among these numbers, 5 showed a zone of inhibition of essential oil, 6 showed a methanolic extract of sweet lime pulp, 7 showed a methanolic extract of sweet lime seed, 8 showed a methanolic extract of sweet lime peel and S showed a zone for standard which was 1% Fluconazole.

Table 2: Samples showed zone of Inhibition (mm) against Fungi.

Tested Organisms	Standard (1% Fluconazole)	Essential Oil	Methanolic Extract of Sweet lime Seed	Methanolic Extract of Sweet lime pulp	Methanolic Extract of Sweet lime peel
Aspergillus niger	5	40	38	20	35
Aspergillus alternata	20	22	18	18	23
Aspergillus terreus	17	17	11	20	20

The comparison of these results also illustrated in the following graph.

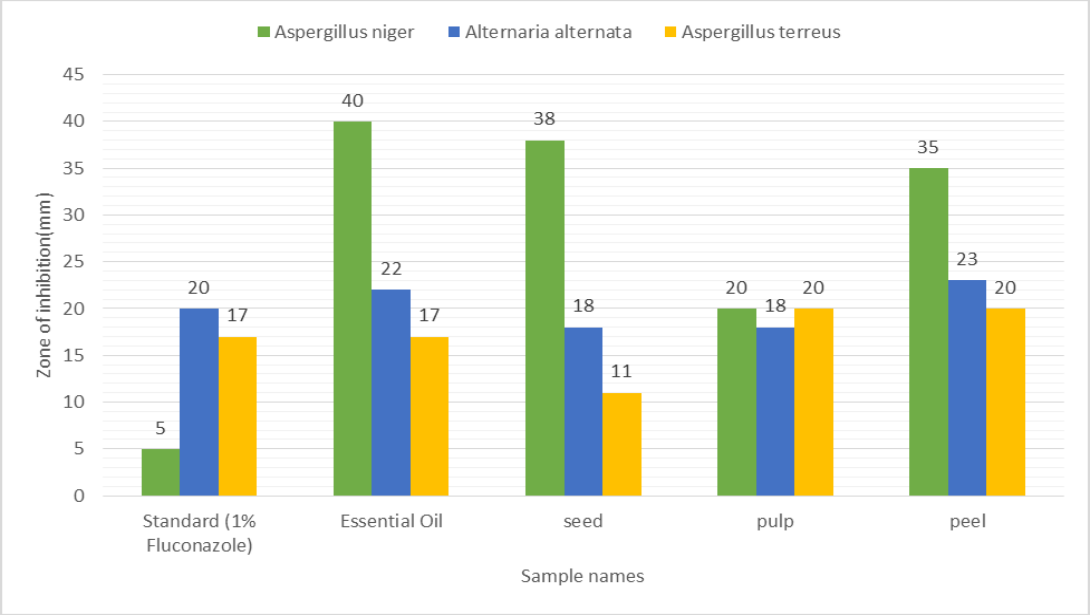

Figure 8: Antifungal activity of essential oil and methanolic extracts.

DISCUSSION

Research showed that peel oil exhibited a maximum zone of inhibition against *Bacillus cereus* (28 mm) and *Bacillus subtilis* (26 mm) followed by *Staphylococcus aureus* (21 mm) after 48 h of incubation at 37°C. while against *Aspergillus niger*, *Aspergillus flavis, Aspergillus fumigates*, and *E. coli* showed 22, 19, 14, and 13 mm zones of inhibition (Javed *et al*., 2013). Essential oils of two citrus fruit including *C. hystrix* and *C. aurantifolia*, showed antibacterial action against *B. cereus, S. aureus*, and *S. typhi* (Methawiriyasilp *et al*., 2003). The results of (Mahmud *et al*., 2009) also exhibited that the highest zone of inhibition against *B. subtilis* was displayed by the peel oil of *C. acida.*

Numerous other investigations have also demonstrated the potent antibacterial properties of α-pinene, limonene, and linalool (Filipowicz et al., 2003; Meccia *et al*., 2007; Yamazaki *et al*., 2004). (Kekuda *et al*., 2009) investigated the antifungal efficacy of steam-distilled peels from three different citrus fruits: *Citrus limetta, Citrus sinensis, and Citrus limon*. They discovered that *C. limetta* was the most effective at inhibiting the growth of the fungi. Further study showed that the antimicrobial property of the essential oil is due to the presence of active components that influence certain metabolic processes of microbial cells (Methawiriyasilp *et al*., 2003). Furthermore, a few components in smaller amounts might also be involved in the oil's antimicrobial activity (Matasyoh *et al*., 2007).

Literature showed that various citrus peels have antibacterial properties (Dhanavade *et al*., 2011; Lawal *et al*., 2013). The strong antibacterial activity of an orange peel fruit extract was demonstrated by (Dubey *et al*., 2011) against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shigella flexineri*, *Bacillus subtilis*, and *Escherichia coli.* The Kagji Lemon peel showed inhibition at 31.25 µg/ml concentration against *S. aureus* on methanolic extract, which inhibits at 31.25 µg/ml concentration. The extracts of Kagja Lemon, South African Malta, and Dargiling Orange on both methanol and ethyl acetate have very high antibacterial activity on B. cereus. The four extracts Elachi Lemon, Batabi Lemon, Egyptian Malta, and China Lemon showed moderate activity against *B. cereus*, where the inhibitory concentration lies between 62.5 µg/ml to 125 µg/ml while China Orange and African Orange extract has weak activity against *B. cereus (*Afroja *et al.*, 2017*).*

The present research showed that by using Streptomycin as a standard essential oil and methanolic extracts of sweet lime peel, pulp, and seed showed maximum percentage inhibition against *Escherichia coli* were 17mm, 9mm, 10mm, and 5mm, *Salmonella Typhi* was 20mm, 8mm, 12mm, and 5mm and *Staphylococcus aureus* were 12mm, 11mm, 12mm, and 8mm respectively. Antifungal activity of essential oil and methanolic extracts of sweet lime seed, pulp, and peel against *Aspergillus niger* was 40mm, 38mm, 20mm, and 35mm, *Aspergillus alternata* were 22mm, 18mm, 18mm, and 23mm and *Aspergillus terreus* were 17mm, 11mm, 20mm, and 20mm respectively and compare these results with *1% fluconazole* as a standard.

Research showed that tangerine peel oil exhibited a maximum zone of inhibition (25mm) against Escherichia coli and *Salmonella Typhimurium* followed by *Aspergillus niger* (19.6mm), whereas the minimum zone of inhibition was shown by *Fusarium solani* (9mm) after 48 hours of incubation at 37Ċ. However, *Aspergillus flavis*, *Aspergillus fumigatus*, *Aspergillus ficuum*, *Staphylococcus aureus*, and *Enterobacter aerogenes* gave 12, 14, 16.6, 11, and 17.8 mm of zones of inhibition respectively after 48 hours of incubation.

Essential oil has been discovered to possess antibacterial characteristics due to its abundance in secondary metabolites, including tannins, terpenoids, alkaloids, and flavonoids (Porter & Wilkins, 1999).

CONCLUSION

The results of our study showed that essential oil and methanolic extract of sweet lime peel show the highest zones of inhibition against *Escherichia coli*, *Salmonella Typhi* and *Staphylococcus aureus*, *Aspergillus niger*, *Aspergillus alternata*, and *Aspergillus terreus* and compare these results with *Streptomycin* and *1% fluconazole* as a standard. Essential oil and peel methanolic extract showed higher activity due to the presence of phenolic content such as limonene, linalool, β-myrcene, and β-citronellol.

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