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Assessment of the Antibacterial Properties of Fruit Peel Extract from *Citrus limon* and *Citrus limetta*

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Abstract

The utilization of plant-derived compounds has emerged as a promising source of bioactive substances with potential antimicrobial properties. Evaluating the antibacterial activity of fruit peel extracts from *Citrus limon* and *Citrus limetta* holds significant promise in exploring their applications against bacterial infections. Despite the common practice of discarding *Citrus limon* peel, it contains numerous bioactive compounds, as does *Citrus limetta* peel. Investigating the antibacterial potential of these extracts, if proven effective, could lead to their utilization as natural antimicrobial agents in pharmaceuticals, food preservation, or as substitutes for synthetic chemicals across various domains. This study aims to assess the antibacterial efficacy of fruit peel extracts from *Citrus limon* and *Citrus limetta* against various bacterial strains, thereby determining their potential as natural antimicrobial agents. *Citrus limon* and *Citrus limetta* peels were ground using a mortar and pestle and then extracted with ethanol. The resultant extract was further diluted to create different concentrations. Antimicrobial assay was (Zone of inhibition) conducted using *Staphylococcus aureus* (SA), *Enterococcus faecalis* (EF), Methicillin-resistance *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Streptococcus mutans* as test organisms. The ethanol extract of C. limon and C. limetta peel extract was found inhibiting all the microbes at a maximum concentration of 100%. Among five different microbial pathogens, the extract the greater inhibition was found against *S. mutans*. In conclusion, the evaluation of antibacterial activity in fruit peel extracts from *Citrus limon* and *Citrus limetta* underscores their potential as natural antibacterial agents. Further investigations are warranted to isolate and identify the specific bioactive compounds responsible for this observed antibacterial activity.

Keywords: *Citrus limon*, C*itrus limetta*, Crude peel extract, antimicrobial activity.

Introduction

The overuse of antibiotics in both humans and animals has led to increased natural antibiotic resistance [1]. To ensure global health security, it is imperative to recommend the correct use of antibiotics and develop new potent antibiotics [2]. Natural resources, especially essential oils (EO), offer promising solutions to this global issue [3,4,5].(Azghar et al., 2023; Dalli et al., 2021; Iseppi et al., 2021) The Citrus genus, which belongs to the Rutaceae family, includes a wide variety of trees cultivated worldwide [6]. Citrus fruits are commonly consumed fresh, used in desserts, or processed into juices and jams. They are renowned for their rich vitamin content, notably vitamin C, and their distinctive flavours [6].

These EO are valued in the pharmaceutical industry, food preservation, perfumery, and cosmetics due to their rich composition of monoterpene hydrocarbons (like limonene),

sesquiterpene hydrocarbons, and their oxygenated derivatives, including aldehydes, ketones, acids, alcohols, and esters [7]. EOs from the Citrus genus are known for their wide range of pharmacological effects, including antioxidant properties [8]. antifungal activity [9]. hypoglycaemia and hypolipidemic activities [10]. as well as neuroprotective effects [11]. However, to the best of our knowledge, only one study has assessed the antibacterial effect of Citrus peel EO specifically against methicillin-resistant Staphylococcus aureus (MRSA) [12]. Citrus peel oils also have potent antioxidant and antibacterial properties [13].

Citrus is a medicinal plant characterized by a diverse range of pharmacological attributes. It ranks among the most extensively cultivated fruit crops globally, with an annual production of 124.3 million tons [14]. *Citrus limon* (L.) Burm. f., commonly known as lemon, is a Rutaceae tree bearing evergreen foliage and yellow edible fruits. While *C. limon* fruit is renowned for its nutritional benefits, its significant biological activities are often overlooked in current phytotherapy and cosmetics [15]. Citric acid, ascorbic acid, minerals, flavonoids, and essential oils are among the natural substances abundant in lemon, endowing it with medicinal properties and rendering it useful in various disciplines such as medicine and food technology [16].

Sweet lime (*Citrus limetta*), also known as "Mosambi," is another popular citrus fruit celebrated for its distinct flavor, fragrance, and taste. While sweet lime is predominantly consumed fresh or in juice form, its non-edible sections, such as peels, constitute a significant source of physiologically active components including phenols, flavonoids, essential oils, carotenoids, organic acids, ascorbic acid, and vitamins. *Citrus limetta*, belonging to the Rutaceae family, enjoys global recognition for its remarkable nutritional and therapeutic properties. Despite the primary use of Mosambi fruits by juice processing firms, the peel and seeds are often discarded. However, citrus wastes, rich in minerals and phytochemicals, can be repurposed as ingredients in medicinal formulations or dietary supplements [17].

The oral cavity harbors a myriad of commensal bacteria, which may serve as reservoirs for antibiotic resistance determinants. *Staphylococcus aureus*, both a commensal and a pathogen, is frequently isolated from the anterior nares as a commensal but can also colonize other anatomical sites, including the oral cavity. *S. aureus* prevalence varies demographically, with reports indicating carriage rates ranging from 24% to 84% among healthy dentate individuals and 48% among denture wearer [18]. *S. mutans*, a prevalent oral streptococcus, plays a pivotal role in oral infections, including periodontitis. Periodontitis, characterized by inflammation and infection of the gingiva and surrounding tissues, often results from biofilm-forming bacteria, posing significant public health challenges in both children and adults.

Enterococcus faecalis, a prominent species in endodontic treatment failure, is implicated in various oral cavity infections. E. faecalis is commonly associated with oral disorders such as caries, endodontic infections, periodontitis, and peri-implantitis, owing to its high resistance to endodontic medicaments and propensity to form refractory biofilms in root canals [19]. The study aims to assess the combined antibacterial efficacy of *Citrus limon* and *Citrus limetta* against select bacterial strains.

Materials and Methods

Extraction

Ten grams each of *Citrus limon* and *Citrus limetta* leaves were ground using a mortar and pestle and extracted with 200 mL of 100% ethanol (EtOH) for two days. The extract was filtered using Whatman No.1 filter paper, and the ethanol was evaporated.

Antimicrobial Assay - Zone of Inhibition (ZOI)

Test organisms included in the study were *Staphylococcus aureus* (SA), *Enterococcus faecalis* (EF), Methicillin-resistant - *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (PA), and *Streptococcus mutans* (SM). Three concentrations (20 mg, 10 mg, and 5 mg) of the crude extract were prepared. Fresh broth suspensions of test organisms were adjusted to 0.5 McFarland Standards. Lawn cultures were made on sterile Mueller-Hinton agar plates, and wells were cut using a sterile agar cutter. Twenty mg, 10 mg, and 5 mg of each test compound were added to respective wells. Plates were incubated at 37°C for 24 hours, and the zone of inhibition were measured and recorded.

Results and Discussion

Compounds that kill or inhibit the development of bacteria locally without being very harmful to adjacent tissues are known as antibacterial active molecules [20]. Previous research suggests that lemon peel exhibits both astringent and antibacterial properties, making it effective against certain skin bacteria. O. sanctum L. (Tulsi) plant extract illustrates antimicrobial efficacy against anaerobic oral microbes. O. sanctum L. (Tulsi) exhibited efficient antimicrobial activity against anaerobic oral microbes proving its potential use as an efficient and standard supplement in the treatment of periodontal conditions [21].

In the present study, ethanol extract of *C. limon* and *C. limetta* extract inhibits all the pathogenic microbes included in the present study. However, the activity was found diminishing upon decreasing the concentration of the extract (**Fig. 1 & 2**). At 100% and 50% concentration, the crude extract was found effective in inhibiting all the microbes except 25% concentration, in which, it has no inhibition against *S. aureus* and MRSA. Among different microbes, the extract has a maximal activity against *S. mutans* and the least effective was

MRSA (**Fig. 1 & 2**). The green synthesis of CaOHAgNPs synthesized from *Andrographis paniculata* and *Ocimum sanctum* yielded an effective nanoparticle preparation that could be used against common oral pathogens as a potential therapeutic agent in the form of root canal irrigant or intracanal medicament in the field of dentistry [22].

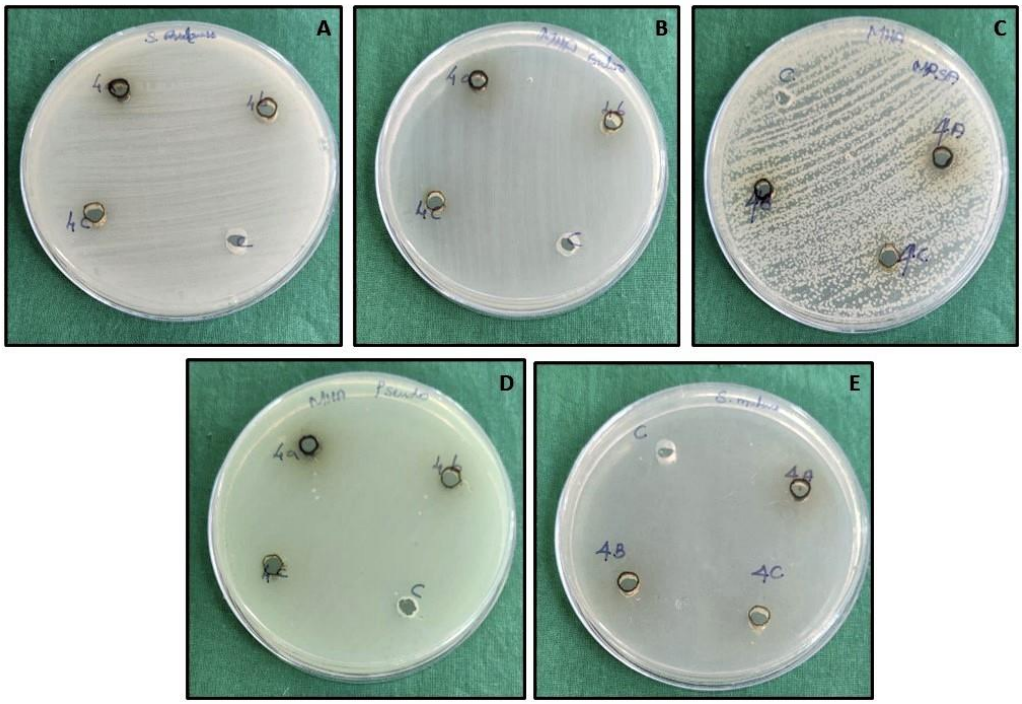

Fig. 1. Petri dishes showing antimicrobial activity of *C. limon* and *C. limetta* extracts as zone of inhibition

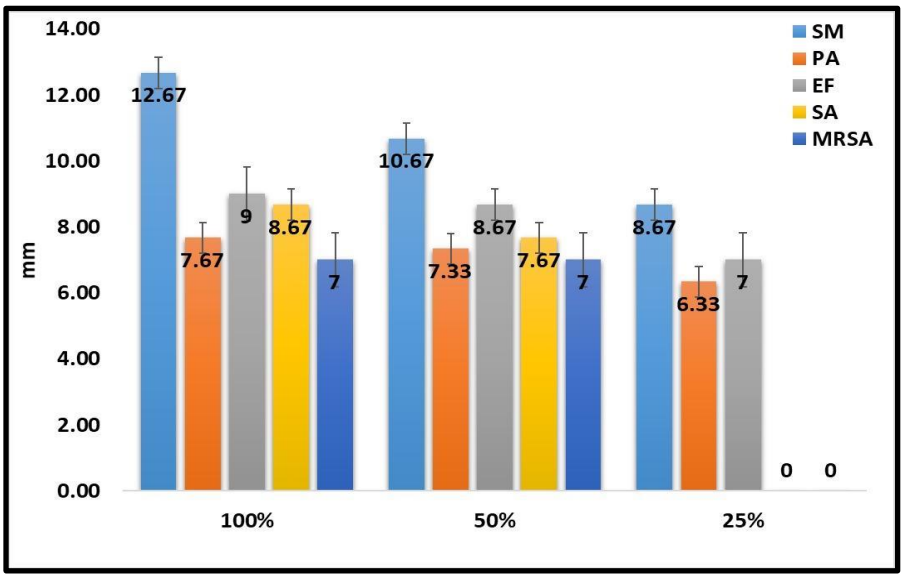

Fig. 2. Bar graph showing the antimicrobial activity of *C. limon* and *C. limetta* extract in three different concentrations as zone of inhibition (mm).

Citrus reticulata peel extracts have also demonstrated antibacterial activity, with the ethanol-soluble fraction showing significant efficacy, indicating their potential as bio preservatives [23]. One of the infectious and major oral health problems affecting mankind since years is dental caries. While Streptococcus mutans bacteria is the main cause of tooth decay, Lactobacilli characteristically cause existing carious lesions to progress [26]. Selenium nanoparticles extracted from Capparis decidua fruit showed good antibacterial activity against lactobacillus species and *E. coli* [24]. However, copper nanoparticles derived from ginger (*Zingiber officinale*) showed a maximum inhibitory zone against oral pathogens [25]. Simultaneously, the nanoparticle possesses antifungal activity when synthesized from tulsi and turmeric [26]. The coconut oil also found to inhibit oral fungal biota including *Candida albicans* [27].

Terpenoids observed in ethanolic extracts is speculated to be involved in membrane disruption by the lipophilic compounds [28]. The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids. These compounds are known to be biologically active and therefore aid the antimicrobial activities of the plants. These secondary metabolites exert antimicrobial activity through different mechanisms. Tannin as observed in *Citrus sinensis* peel extract have been found to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis [29].

Conclusion

The antibacterial activity observed in fruit peel extracts from *Citrus limon* and *Citrus limetta* underscores their potential as natural antimicrobial agents. Further research is warranted to elucidate the specific bioactive compounds responsible for this activity.

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