

Studies on the antibacterial activity of non-aqueous extract of different species of *citrus* fruit seeds against *Lactobacillus casei*

Uma Maheswari Kolipaka^{1*}, A. Sumana Sri^{2*}, Sk. Karishma³, G. Vinay Kumar Reddy⁴, B. Devi Somya Sri⁵, R. Gowri Prasad Singh⁶, I. Siva Sudhakar Reddy⁷

¹Associate Professor, Dept. of Ph. Biotechnology, A.M Reddy Memorial College of Pharmacy, Petlurivaripalem, Narasaraopet, Palnadu (dt), A.P-522601

^{2,3,4,5,6,7}IV/IV B.Pharm Students, A.M Reddy Memorial College of Pharmacy, Narasaraopet

Received: 21st Sept, 25; Revised 12th Oct, 25, Accepted: 10th Nov, 25; Available Online: 21th Nov, 25

ABSTRACT

Citrus fruits, including lemons, oranges, mosambi (sweet lime), and pomelos, possess a diverse range of applications spanning from culinary uses to practical household tasks. Lemons are well-known as a rich source of vitamin C, valued for their sharp taste and immune-boosting properties. Their applications extend to cooking, baking, cleaning, and even skincare routines. Oranges are primarily utilized as a food source, offering various health advantages. They are consumed fresh, processed into juice, and incorporated into numerous food preparations like jams, marmalades, and salads. Their significant nutritional content, particularly vitamin C, contributes to a healthy diet. Mosambi provides several health benefits and can be used both internally and externally. It is a good source of vitamin C, aiding in digestion, strengthening immunity, and potentially lowering the risk of certain diseases. Additionally, mosambi has applications in skin brightening and hair care. Pomelos are versatile fruits enjoyed as snacks or desserts and integrated into various recipes. They can be eaten raw or used in salads, marmalades, and candied forms, as well as juiced or added to smoothies.

The current research explored the antibacterial potential of a non-polar solvent extract from the seeds of four citrus fruit varieties against *Lactobacillus* species. An ethanol and diethyl ether extract was prepared through a 72-hour soaking process, followed by separation of its components using thin-layer chromatography (TLC) and liquid column chromatography. Phytochemical analysis of the isolated fractions revealed the presence of phenols, flavonoids, and terpenoids. Antibacterial activity was evaluated using the cup plate method, which showed a 15mm inhibition zone for the initial extract against the tested *Lactobacillus* species. These findings indicate that the non-polar extract of citrus fruit seeds contains active compounds with antibacterial properties, suggesting a need for further research into its potential use in developing new antimicrobial agents.

Keywords: Antimicrobial, Pomelo, diethyl ether extract, lemon, mosambi, orange

INTRODUCTION

The use of plant-based medicine is widespread and continues to grow (1). Plants have been traditionally used to treat various infections and illnesses due to their inherent defense compounds against insects, herbivores, and microorganisms (2). Citrus fruits are a group of plants recognized for their significant medicinal value (3). The therapeutic effectiveness of citrus fruits like lemon and grapefruit is supported by the presence of diverse polyphenolic flavonoids, which have demonstrated antibacterial, antifungal, and antioxidant activities (4, 5). Sweet oranges contain vitamin C, fiber, and other bioactive compounds, including carotenoids and phenolic compounds [7]. Edible orange juice exhibits antioxidant properties [8], attributed to its high content of vitamin C, flavonoids, and phenolic compounds. Generally, antioxidants reduce oxidative stress, a common factor in health disorders, supporting Crowell's suggestion [9] that these bioactive compounds in sweet orange juice may lower the risk of cancers and many chronic diseases. Citrus limonum (lemon) contains esculetin, a bioactive

compound that improved health markers in rats [10], while sweet orange peel essential oils have shown antiseptic, analgesic, and anti-inflammatory effects [11, 12]. Given the increasing human health challenges, potentially arising from food and food additives [13, 14], ongoing research is essential to scientifically validate the use of plants, plant parts, fruits, and fruit byproducts. This study aimed to extract, isolate, and identify the antibacterial effects of self-prepared ethanol and diethyl ether extracts from citrus fruit seeds against *Lactobacillus* species and to compare these effects with erythromycin.

MATERIAL AND METHODS:

The research methodology involved four key stages: extraction, TLC and column chromatography, phytochemical screening, and antimicrobial activity testing. Each stage is detailed below: An ethanol and diethyl ether (1:1) extract was prepared from the seeds of commercially available lemon (*Citrus limon*), mosambi (*Citrus limetta*), oranges (*Citrus x sinensis*), and pomelos (*Citrus maxima*). The seeds were air-dried in the shade and

*Author for Correspondence: fromuma7@gmail.com

then coarsely ground into a powder. This powdered sample was wrapped in filter paper and subjected to extraction with a 70% ethanol and diethyl ether mixture in a conical flask for 72 hours. Following this, the crude extract was obtained by filtration to remove solid residues. The liquid extract was then analyzed using TLC and further separated into different fractions using column chromatography.

The bacteria utilized in this study, *Lactobacillus leissi*, were sourced from homemade curd. These microorganisms are standard strains commonly employed in antimicrobial activity assays. The antimicrobial activity testing was conducted using the agar diffusion method, following the Shinji Miyadoh method [6]. The test microorganism was evenly spread on nutrient agar plates. Sterile blank antibiotic discs were then placed on the agar surface, and 25 to 50 microliters of the test solutions were applied to these discs. The same volume of distilled water (negative control) and a broad-spectrum antibiotic (positive control) were also tested. The agar plates were then incubated for 24 hours. All experiments were performed in triplicate, and the antimicrobial activity was expressed as the average diameter (in millimeters) of the clear zones of growth inhibition observed around the discs.

RESULTS AND DISCUSSION:

Seeds from *Citrus limon*, *Citrus limetta*, *Citrus maxima*, and *Citrus x sinensis* were collected, air-dried, and mixed before being coarsely powdered and extracted using ethanol and diethyl ether as solvents. Following the 72-hour maceration process, the extract was analyzed using TLC, which indicated the presence of three or four components. Column chromatography was then employed to separate these components. Phytochemical screening was performed on the separated fractions, revealing the presence of phenols, flavonoids, and terpenoids. The total weight of the dried seeds was 51.36 grams, which were extracted with 300 ml of 70% ethanol and 300 ml of diethyl ether for 72 hours. Subsequent evaporation of the solvents by distillation yielded 43 mL of concentrated seed extract. TLC analysis confirmed the presence of different compounds in the ethanol and diethyl ether extract, and column chromatography successfully separated the extract into various fractions. Phytochemical analysis of these fractions confirmed the presence of phenols, flavonoids, and terpenoids (Table 1). Antimicrobial activity testing was then conducted on three fractions of the extract using the cup plate method, revealing different zones of inhibition. Erythromycin sulfate was used as a standard drug in this microbiological assay. The results of the microbiological assay are presented in Table 2.

Table 1: Phytochemical screening of ethanol and diethyl ether extract of mixed seeds.

S.No	Phytochemical/standard drug	Zone of inhibition in mm
1.	Phenolic fraction (50UI)	13.4
2.	Flavonoids fraction (50UI)	11.8
3.	Terpenoids fraction (50UI)	12.5
4.	Standard drug- Erythromycin sulphate (50UI)	18.2



Figure 1: Zone of Inhibition of Flavonoid Phenolic and terpenoid content of Citrus fruits seeds extract against *Lactobacillus leissi* respectively.

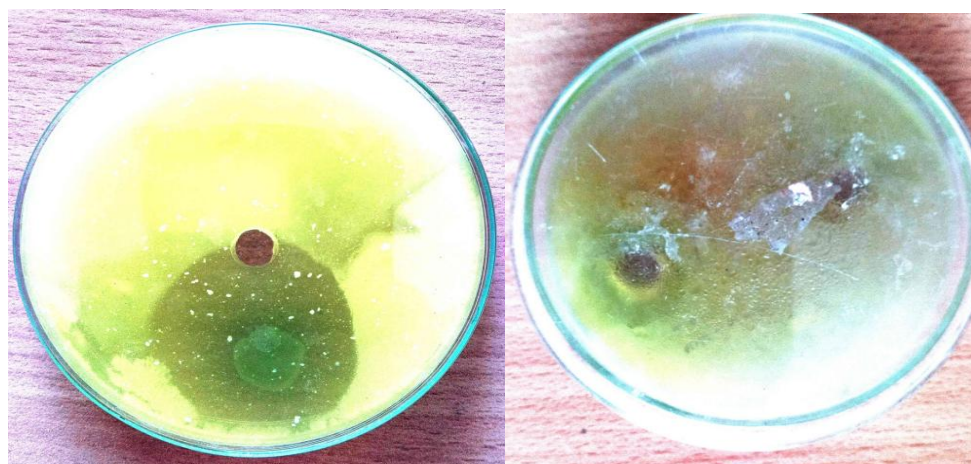


Figure 2: Zone of Inhibition of standard drug and Phenolic portion of Citrus fruits seeds extract against *Lactobacillus leissi* respectively.

Table 2: Phytochemicals

S. No.	Phytochemicals	Test results
1.	Phenols	++++
2..	Flavonoids	+++
3.	Terepenoids	+++
4.	Carbohydrates	
5.	Glycosides	
6.	Alkaloids	

CONCLUSION:

The extraction of an ethanol and diethyl ether extract from the seeds of Citrus limon, Citrus limetta, Citrus x sinensis, and Citrus maxima was successfully achieved. The antimicrobial activity of different components of this extract against *Lactobacillus leissi* was demonstrated and compared to erythromycin. This study concludes that the ethanol and diethyl ether extract from various citrus fruit seeds possesses antimicrobial activity. Further in-depth investigation of these components using advanced spectral analysis and microbiological assays against a broader range of microorganisms could lead to the development of novel antibiotic compounds.

REFERENCES

- Atolani, O., Omere, J., Otuehere, C.A and Adewuyi, A. (2012). Antioxidant and cytotoxicity effects of seed oils from edible fruits. *Journal of Acute Disease*: 130-134 (doi:10.1016/S2221-61899(13) 60073-6).
- Baghurst K. (2003). The health benefits of citrus fruits. CSIRO Health Sciences and Nutrition full report. ProjectNo: CT02057. Horticulture Australia Ltd., Sydney, Australia.
- Ben Gem J.B. (1967). Citrus products: chemical composition and chemical technology. Inter-science publishers.
- Braverman, J.B (1949). Citrus products: chemical composition and chemical technology. Inter-science, New York.
- Guimaraes, R., Barros, L., Barreira, J.C., Sousa, M.J., Carvalha, A.M and Ferreira, I.C.F.R (2010). Targeting excessive free radicals with peels and juice of citrus fruits grapefruits, lemon, lime and orange. *Food and Chemical Toxicology*, 48(1):99-106.
- Gupta, C., Garey, A.P., Uniyal, R.C and Kumari, A. (2008). Antimicrobial activity of some herbal oils against common food borne pathogens. *African Journal of Microbiology Research*, 5(2):258-261
- Hegazy, A.E. and Ibrahim, M.I. (2012). Antioxidant Activities of Orange Peel extracts. *World Applied Science Journal*, 18(5): 684-688.
- Hiroyuki, T., Tetsuro, K., Masaguki, A.K., Fusao, O. (2006). Antimicrobial Activity of Citrus Fruits juice against vibro species, 52(2): 157-160.
- H. O. Edeoga, D. E. Okwu and B. O. Mbabie. *African Journal of Biotechnology*, pp. 4: 685-688(2005).
- M. A. Habib, M. A. Hammam, A. A. Sakr and Y. A. Ashoush. *Journal of American Oil Chemis' Society*, no. 63, pp. 1192-1197(1986).
- A. A. Ordonez, J. G. Gomez, M. A. Vattuone and M. I. Isha. *Food Chemistry*, no. 97, pp. 452-258(2006).
- M. A. Ebrahimzadeh, S. J. Hosseinimerh, A. Hamidinia and M. Jafari. *Pharmacologyonline*, no. 1, pp. 7- 14(2008).
- M. A. Ebrahimzadeh, F. Pourmorad and A. R. Bekhradnia. *African Journal of Biotechnology*, no. 32, pp. 43- 49(2008).
- S. M. Nabavi, M. A. Ebrahimzadeh, S. F. Nabavi, A. Hamidinia and A. R. Bekhradnia. *Pharmacology Online*, no. 2, pp. 560-567(2008).