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Evaluation of the phytochemical constituents and antibacterial efficacy of *Limonia acidissima* leaves on bacteria from spoiled tomatoes

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Abstract

The study explores the phytochemical composition and antibacterial properties of *Limonia acidissima* leaf ethanolic extract, which has been found to extend the shelf life of tomatoes. FTIR spectroscopy revealed the presence of bioactive compounds with potential antioxidant and antibacterial properties. GC-MS analysis confirmed the presence of phytochemicals, including alkaloids, steroids, flavonoids, fatty acids, and esters, supporting the plant's traditional medicinal usage. Microbiological analysis of spoiled tomato samples identified four bacterial strains, and the ethanolic extract showed significant inhibitory effects against them in a dose-dependent manner. Applying the extract at 100% concentration effectively prolonged tomato shelf life up to 15 days, suggesting its therapeutic and preservative potential as a natural food preservation and safety alternative.

Keywords: *Limonia acidissima,* FTIR, GC-MS, Antibacterial activity, Shelf life of Tomatoes

Introduction

Limonia acidissima L. (wood apple) is a member of the family, *Rutaceae* and is a religious tree planted in temples and gardens known for its medicinal and processing properties which is a moderate sized deciduous tree grown throughout India **Lamani & Murthy, 2025**. It grows upto 9 meters tall and has pinnate leaves with 5-7 leaflets. The fruit, a berry, is 5-9 cm in diameter and can be sweet or sour. The fruit has a hard rind and sticky brown pulp and small white seeds. The woody, rough fruits are used as a substitute for bael in diarrheoa and dysentery. The bark and leaves are used for vitiated conditions, while the fruits are used for tumors, asthma, wounds, cardiac debility, and hepatitis **Khatun & Sen, 2024.** The leaves have hepatoprotective activity, while the fruit contains flavanoids, glycosides, saponins, tannins, coumarins, and tyramine derivatives. The leaves contain stigmasterol, psoralen, bergapten, orientin, vitedin, saponarin, tannins, and an essential oil. The fruit shells contain antifungal compounds. The stem bark of the plant has yielded (-)- (2S)-5,3'-dihydroxy-4'-methoxy-6",6"dimethyl chromeno-flavanone, along with several compounds with antimicrobial activity. The leaves have traditionally been used for diarrhoea, wound healing, and boils, indicating their antimicrobial activity **Sujitha & Venkatalakshmi, 2021**.

Wood apple has been used in traditional medicine for thousands of years, with both ripe and unripe fruits having therapeutic properties **Parvez & Sarker, 2021**. The phytochemicals and minerals in the fruit have been used to treat various ailments, including dysentery, diarrhoea, asthma, wounds, tumors, hepatitis, and cardiac debility **Amardeepa & Vijayakumar, 2025**. Ripe fruit also helps treat liver disease and heart problems, and lowers cholesterol levels. Juice of wood apple with warm water and sugar is recommended for detoxification and removing toxins from the body. Children can use juice mixed with milk and sugar for biliousness and intestinal problems **Lamani & Murthy, 2025**. The essential oil of crushed leaves is used to treat itching and improve digestion. Wood apple leaves contain high tannins, which are effective against peptic ulcers.

The leaves of the plant are known for their medicinal properties, including treating breast cancer, uterus cancer, infertility, progesterone deficiency, flu, and respiratory disorders **Khatun & Sen, 2024**. They also have traditional uses in snake bites and against bacterial pathogens. The pulp, with a low fat content, is beneficial for overweight individuals. The fruits are astringent, carminative, and hepatoprotective. The seeds are used to treat heart diseases. The spine of the tree is a remedy for menorrhagia. The bark contains coumarins and is applied to venomous wounds. The gum has demulcent, constipating, anti-diarrheal, and anti-haemorrhoidal properties.

Tomatoes are a popular berry plant from the nightshade family, originating in South Africa and brought to the Philippines and Malaya in the 1650s. They are a delicate perennial plant that grows up to 1-5 meters tall and has a fruit structure that emerges from the ovary after fertilization **Sattar *et al.,* 2023**. The fruit is a true fruit consisting of seeds and moisture embedded in a gelatinous mass of lobed cavities. The color of the fruit ranges from green at tender age to yellow/orange or red at maturity, depending on the predominant pigment lycopene or B-carotene **Mohd Hassan *et al.,* 2019**. Tomatoes are a popular fresh fruit worldwide due to their balanced diet, rich in vitamins, carbohydrates, minerals, trace elements, and fiber. Tomato fruits contain 4.3% carbohydrates, 1% protein, 0.1%, 94% water, fat, 0.6% fiber, and vitamins **Suman *et al.,* 2023**.

Tomatoes, a popular fruit in the *Solanaceae* family, can be eaten raw or cooked. They contain Vitamin A, Vitamin C, carbohydrates, proteins, lipids, fibers, and potassium. Tomatoes are rich in lycopene, providing health benefits. However, their high water content increases susceptibility to bacterial action. Tomatoes have a lower sugar concentration than other fruits, making them less sweet **Sun *et al.,* 2023.**

Tomatoes can deteriorate due to microbial degradation during storage, shipping, and processing, lowering their market value and nutritional quality **Umeohia & Olapade, 2024.** Contaminations with mycotoxins, which form aflatoxins in humans, can render tomatoes unfit for consumption, leading to food poisoning. Research has identified bacteria and fungi linked to tomato rotting, including *Bacillus subtilis, Klebsiella, aerogenes, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis,* and *Staphylococcus aureus* **Opara & Okoronkwo, 2024**. In Benin City and Lagos State, significant amounts of *Staphylococcus spp., Bacillus spp.,* and *Escherichia coli* were found. *Rhizopus sp.* has also been linked to tomato rotting **Ekundayo *et al.,* 2024**.

Tomatoes are perishable due to diseases, improper handling, and environmental conditions, causing significant economic losses **Firdous, 2021.** In Nigeria, up to 60% of harvested tomatoes are lost due to rot, poor storage, transportation, and marketing issues. Tomato storage requires temperature, with 12°C being the optimal compromise for slowing ripening without cellular damage. Tomatoes offer nutritional benefits like lycopene, vitamin C, potassium, and fiber, and their preservation is essential for food security. Identifying spoilage-causing microbes can help reduce postharvest losses and ensure better-quality tomatoes **Sharma *et al.,* 2024.**

Materials and Methods

Collection of Limonia acidissima and Tomato Samples

Limonia acidissima leaves were taken in Namakkal, Tamil Nadu, India, in latitude 11.4803730 and longitude 78.4089070. A tomato sample was acquired at a local market in Kallakurichi, Tamil Nadu, India. They were taken to the microbiology laboratory at AVS College of Arts and Science (Autonomous) Salem for microbiological investigation.

Preparation of Solvent Extract

The extraction of plant leaves was conducted using a cold exhaustive maceration method, following the method suggested by **Vongsak *et al.,* (2014)**. The collected leaves were air-dried and ground into a coarse powder. The powder was then dispensed into a conical flask and added to 200 ml of ethanol. The flasks were allowed to macerate for 24 hours at room temperature with intermittent shaking to facilitate proper dissolution and extraction. The contents were then filtered through muslin cloth and Whatman filter paper, and the final filtrate was collected in a petridish. The final filtrate was evaporated on a water bath at 30^0C, resulting in a solvent-free, semi-solid aqueous extract. The final extract was mixed with an equal ratio of (1:1) ethanol to obtain the final stock solution.

Structural characterization of plant extract

FTIR spectra analysis

The UV-1800 spectrophotometer from Shimadzu UV-vis was used to record the absorption spectra of the extracted dye in the wavelength range of 400-700 nm. The infrared spectra of the Fourier transformations were recorded in the wave number range of 4000 to 400 cm−1 using the Bruker ALPHAII FTIR spectrometer, which was equipped with an ATR (Attenuated Total Reflection) sample attachment **Ferreira *et al.,* 2020.**

GC-MS analysis

The ethanolic extract of Limonia acidissima leaf was analyzed using gas chromatography with mass spectrometry (GC-MS/MS) using thermo GC-trace ultra ver: 5.0, thermo ms DSQ II equipment. The tr 5-ms capillary standard non-polar column was installed. The electron ionization system had an ionization energy of 70 eV, and 99.9% helium gas was used as the carrier gas. The oven temperature was set to 40°C for one minute, then raised to 150°C, 3°C, and 300°C for ten minutes. The injector temperature and volume were 250°C and 1μL, respectively. The GC-MS took approximately 37 minutes to run.

Tomato Sample Processing

Bacteriological examinations were conducted using standard methods for aerobic bacteria. Each tomato sample was crushed, transferred to a test tube containing sterile distilled water, and diluted to 10-7 dilution factors **Sola *et al.,* 2022.** The diluted dilutions were then plated on Nutrient agar and selective and differential media (Salmonella shigella agar and MacConkey agar). The plates were inverted and incubated aerobically at 37°C for 24 hours, after which they were examined for growth.

Isolation and Characterization of Bacterial isolates

Bacteria colonies on media plates were sub cultured on freshly prepared nutrient agar plates until pure colonies were obtained. Cultural characteristics such as colony color, shape, pigmentation, and opacity were observed on selective media, followed by Gram staining and biochemical tests. Discrete bacteria colonies were cultured on double strength nutrient agar slants and incubated at 37°C for 24 hours. Growth was observed, and the slants were stored in the refrigerator to preserve the bacterial isolate. The isolate on the slant was then sub cultured on freshly prepared nutrient agar.

Determination of Antibacterial Activity

Well diffusion method

Mueller Hinton Agar (MHA) was used to grow bacterial cultures. 100 microlitres of 5-6 hours old bacterial cultures were spread evenly over the agar surface using an L-loop spreader. Wells of 8mm diameter were punched into the agar. Five similar wells were punched out, with one serving as a positive control and the other in series with different concentrations of extracts. Streptomycin discs were placed in the central position, and the first well was added with distilled water as a negative control, and the other wells added with 50 microlitres (µl) of increasing concentrations of extract i.e 25, 50, 75 and100 mg/ml in respective wells. The plates were incubated at 37^0C for 18-24 hours, and the size of the zone of inhibition was measured using a zone scale reader provided by Hi-media.

Determination of shelf life extension of tomato fruits

Five healthy tomatoes were used to evaluate the preservative effect of *Limonia acidissima* ethanolic extract. One tomato served as an untreated control, while the others were treated with varying extract concentrations (25%, 50%, 75%, and 100%). After air-drying, all tomatoes were stored at room temperature and monitored regularly for spoilage indicators such as texture, colour changes, and weight loss. The study compared treated samples to the control to assess the extract's effectiveness in preserving tomato quality **Okolo *et al.*, 2022.**

Results

Physical appearance and consistency of plant extract

The processing of *Limonia acidissima* involves collecting fresh green leaves with a smooth texture, which are then air-dried to a light to dark greenish-brown shade and ground into a fine to coarse powder with a dry, free-flowing consistency. The powdered leaves undergo ethanol maceration, producing a dark green to brownish liquid extract that is filtered for purity. After evaporation, a semi-solid, thick, and slightly sticky extract is obtained, which is then mixed with ethanol to form the final stock solution, appearing dark green to brown with a smooth texture and slightly viscous consistency.

FTIR spectra analysis

The FTIR spectrum analysis reveals two significant absorption peaks at 719.87 cm^{-1} and 3356.53 cm^{-1}, corresponding to distinct functional groups shown in Table 1. The lower wavelength at 719.87 cm^{-1} is associated with C-H bending vibrations, specifically the out-of-plane bending of C-H bonds in alkyl groups, particularly methylene ($-CH_2-$) groups. This typically indicates the presence of alkyl chains in the sample. In contrast, the higher wavelength at 3356.53 cm^{-1} corresponds to O-H stretching vibrations, a characteristic absorption for alcohols and phenols. The broad nature of this peak suggests the involvement of hydrogen bonding within the O-H group, which is a common feature in compounds with alcohol or phenol functional groups.

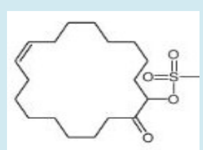
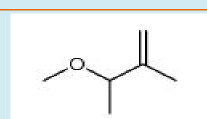
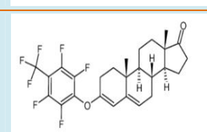
Table 1: FTIR Spectral analysis

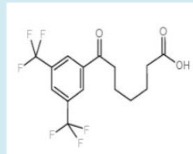
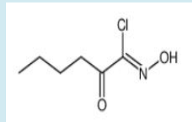
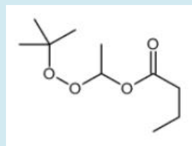
S.NO	WAVE LENGTH	FUNCTIONAL GROUP	STRETCH /BEND
1.	3356.53	Phenols	Stretch
2.	2919.46	-C-H(Alkenes)	Stretch
3.	2853.76	Carboxylic acid	Stretch
4.	2356.71	C≡N(nitrile)	Stretch
5.	1725.40	Ketones	Stretch
6.	1451.16	Alkanes	Bend
7.	1371.18	C-O (Ether)	Bend
8.	1162.64	Alkyl amine	Stretch
9.	719.87	Alkylhalides	Bend

GC-MS analysis of ethanolic leaf extract of *Limonia acidissima*

The ethanolic leaf extract of *Limonia acidissima* was subjected to GC-MS which was carried out by an instrument Thermo GC-trace Ultra Ver: 5.0, Thermo MSD SQ II equipment and quantitatively analysed for the identification of potential bioactive phytochemical compounds. In this graph nearly 7 retention peaks are separated based on the retention times of GC reference were given to three decimal places. There are two different peaks were observed in the interpreted graph as long and short sharp peaks. Among the 7 peaks the compounds observed were listed in the table 2.

Table 2: GC-MS Analysis

S.NO	RT	NAME OF THE COMPOUND	STRUCTURE	MOLECULAR FORMULA	M. W	Biological Activity
1.	05.32	6,7-(Isopropylidenedioxy)-3-(triisopropylsiloxy)-8-oxabicyclo[3.2.1]oct-3-ene		C19H34O4S	358.54	Antimicrobial
2.	11.39	3-Methoxy-2-methyl-1-butene		C6H12O	100.16	Antioxidant
3.	13.41	androstenedione-perfluorotolyl-enol ether		C26H25F7O2	502.46	Hormonal regulator
4.	20.35	Nonahexacontanoic acid		C70H140O2	981.94	Antimicrobial, Antioxidant, Anti-inflammatory

5.	25.01	1-Acetoxy-1-[4,s7-bis(trifluoromethyltetr aicyclo[4.3.0.0(3,5).0(4,9)]non-7-en-9-yl]ethanol isomer		C15H14F6O3	356.26	Antiviral
6.	28.39	4-(2-chloroethyl)morpholin-2-one		C6H10ClNO2	163.60	Antimicrobial, Antifungal, Anticancer
7.	31.41	(R)-Ethyl 4-t-butoxy-3-hydroxybutanoate		C10H20O4	204.26	Antimicrobial, Antioxidant, Anti-inflammatory

Bacterial Characterization from Spoiled Tomatoes

Bacterial isolates from spoiled tomatoes were identified based on distinct cultural, morphological, and biochemical characteristics. *Staphylococcus* spp., *E. coli*, *Salmonella* spp., and *Klebsiella* spp. were confirmed using selective media and biochemical tests. Colony appearances on specific agars and Gram staining results supported their identification, highlighting the presence of foodborne pathogens in the spoiled samples.

Antibacterial activity

Antibacterial activity of the ethanolic extract of the *Limonia acidissima* against the pathogenic organisms such as *Staphylococcus spp., E. coli, salmonella spp.* and *Klebsiella spp.* was assessed by measuring the diameters of the zone of inhibition. In the various concentrations of ethanolic extract of *Limonia acidissimia* to the concentration of 75 & 100 % exhibited maximum zone of inhibition against *Staphylococcus sp.* (7.4& 8.2) and the concentrations of 50& 75% exhibits the maximum zone of inhibition against the *E.coli* (8.1&7.2), where as the *Salmonella* and *Klebsiella spp.* exhibits the maximum of zone of inhibition in the concentration in 50& 100 % (7.0& 6.9) (7.8 & 8.1) which has shown in the Table 3. (Fig. 1)

Table 3: Antibacterial activity

Name of the Bacterial strain	Control	Ethanolic Extract(%)			
	Streptomycin	**25**	**50**	**75**	**100**
Staphylococcus spp.	14.5	7.0	6.3	7.4	8.2
E.coli	15.3	6.2	8.1	7.2	6.0
Salmonella spp.	15.9	6.0	7.0	5.4	6.9
Klebsiella spp.	9.0	6.5	7.8	7.1	8.1

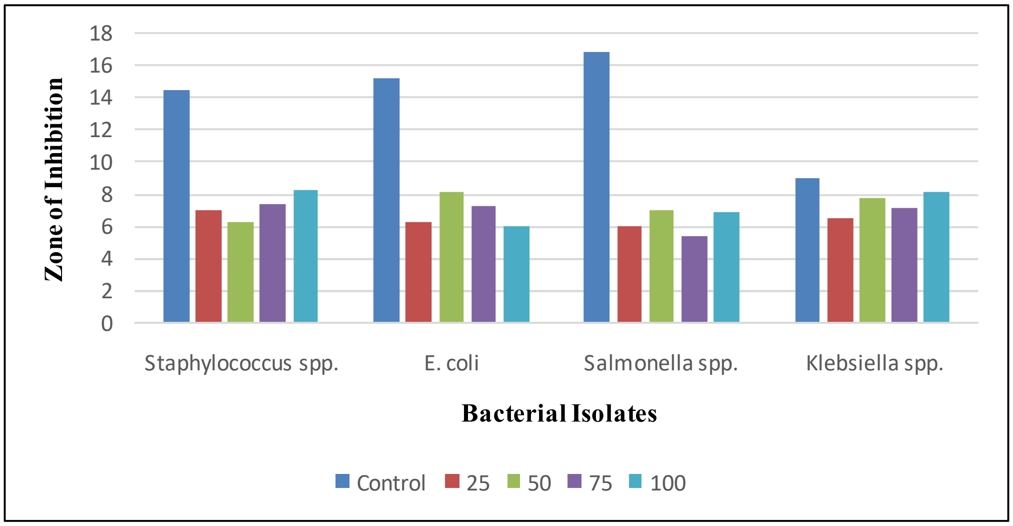


Figure 1: Graphical representation of Antibacterial activity

Shelf life extension of tomato fruits using ethanolic leaf extract of *Limonia acidissima*

The shelf life of tomato samples can be extended using the ethanolic extract of *Limonia acidissima* leaves, with higher concentrations showing increased effectiveness. The treated samples remained fresh for up to fifteen days, as shown in the Table 4. The graphical representation of treatment of the tomatoes where shown in Fig. 2.

Table 4: Treatment of Tomatoes

Samples	Untreated sample	Different concentrations of plant extract (µl/ml)			
		25	**50**	**75**	**100**
TS1	4 days	6 days	7 days	9 days	12 days
TS2	4 days	6 days	8 days	11 days	13 days
TS3	4 days	6 days	7 days	11 days	15 days

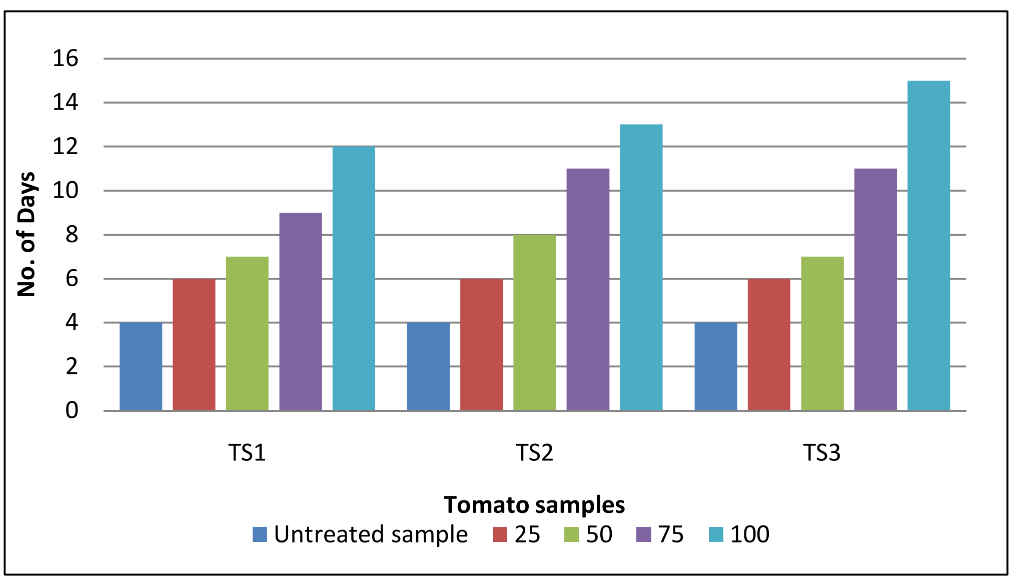


Figure 2: Diagrammatic representation of Shelf life extension of tomato fruits using ethanolic leaf extract of *Limonia acidissima*

Discussion

The phytochemical analysis of *Limonia acidissima* conducted in the present study revealed a diverse range of bioactive compounds, including phenols, carboxylic acids, ketones, alkenes, alkyl amines, and alkyl halides, as identified through FTIR analysis. A comparative review with **Raman Amardeepa and Poongodi Vijayakumar (2025)** showed similarities in the identification of carboxylic acids, ketones, and alkyl halides, while our study uniquely reported the presence of phenols and alkenes. This variation may result from differences in extraction methods and environmental factors, indicating the plant's broad phytochemical diversity. These functional groups are linked to the plant's medicinal and antimicrobial properties, underlining the relevance of FTIR analysis in identifying therapeutic compounds.

GC-MS analysis in the present study further confirmed the presence of alkaloids, steroids, fatty acids, and phenols, supporting the findings of earlier researchers like **Hoa. Ma *et al.,* (2024)**, who reported compounds such as stigmasterol and alpha-pinene. Similarly, **Punitha *et al.,* (2022)** identified alkaloids, phenolics, and terpenoids, aligning with our findings while also indicating slight variations such as the detection of esters in our study. These differences are again attributed to geographic and methodological influences. The consistent presence of bioactive compounds in all studies highlights the medicinal and therapeutic potential of *Limonia acidissima*, particularly in antimicrobial and antioxidant applications.

Microbiological investigations revealed that *Staphylococcus spp.*, *Salmonella spp.*, *Klebsiella spp.*, and *E. coli* were the dominant spoilage organisms isolated from tomatoes, consistent with the findings of **Sivalingam *et al.*, (2024)**. **Ajogwu *et al.,* (2023)** also reported similar bacteria in semi-spoiled tomatoes, emphasizing the public health risk posed by foodborne pathogens. The detection of both Gram-positive and Gram-negative organisms reinforces the need for effective microbial control strategies to ensure food safety. These studies collectively stress the importance of monitoring spoilage microbes in fresh produce and adopting appropriate preservation techniques.

The antibacterial activity of *Limonia acidissima* extract demonstrated a clear dose-dependent inhibitory effect against spoilage bacteria, with higher concentrations showing stronger antimicrobial action. While *Staphylococcus spp.*, *Salmonella spp.*, *Klebsiella spp.*, and *E. coli* were sensitive to the extract, *Vibrio spp.* showed resistance at 100 mg/mL. These results align with Ahmad **Shobrun Jamil *et al.,* (2019)**, who also reported effective antibacterial activity of the plant. The presence of alkaloids, phenolics, and flavonoids likely contributes to this inhibitory effect, supporting its traditional use in treating infections. Overall, the findings validate *Limonia acidissima* as a natural antimicrobial agent suitable for food preservation and potential pharmaceutical applications.

In our study, the shelf life of tomatoes was successfully extended up to 15 days using *Limonia acidissima* leaf extract, demonstrating its strong preservative potential. This finding is in concordance with the previous research by **Okolo *et al.,* (2022)**, which reported the effectiveness of medicinal plants in prolonging tomato freshness. The use of natural plant-based preservatives is a promising alternative to synthetic agents. These results support the growing interest in eco-friendly methods for food preservation and safety.

Summary

The study highlights the antimicrobial and preservative potential of *Limonia acidissima* leaf ethanolic extract. FTIR and GC-MS analyses confirmed the presence of bioactive functional groups and phytochemicals such as phenols, alkaloids, flavonoids, and fatty acids, indicating therapeutic and antimicrobial properties. Four bacterial strains- *Staphylococcus* spp., *Salmonella* spp., *Klebsiella*, and *E. Coli*- were identified as agents of tomato spoilage through cultural, morphological, and biochemical methods. The extract demonstrated strong antibacterial activity against all isolates, with inhibition increasing in a

dose-dependent manner. Furthermore, application of the extract at 100% concentration significantly extended the shelf life of tomatoes up to 15 days by reducing microbial spoilage. These findings support the use of *Limonia acidissima* as a natural antimicrobial agent for food preservation and safety.

Conclusion

The study demonstrated that *Limonia acidissima* leaf extract contains bioactive compounds such as alkaloids, flavonoids, esters, and fatty acids with notable antibacterial activity against spoilage bacteria like *Staphylococcus* spp., *Salmonella* spp., *Klebsiella* spp., and *E. coli*. The antibacterial effect was dose-dependent, with higher extract concentrations showing stronger inhibition. These findings highlight the extract's potential as a natural preservative to extend the shelf life of tomatoes and possibly other perishable foods. Further research is needed to isolate the specific active compounds and explore practical applications in food preservation and safety.

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