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Physicochemical Characterization and Phytochemical Screening of *Citrus aurantifolia* Lime Peel Ethanolic Extract from Kediri as Antibacterial Agent

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Abstract

Citrus aurantifolia (Christm.) Swingle lime peel, widely discarded as agricultural waste in Kediri, East Java, Indonesia, is a promising source of bioactive compounds for antibacterial applications. Quality standardization of herbal materials is prerequisite for reproducible pharmacological activity, yet comprehensive physicochemical and microbiological characterization of lime peel simplicia from this region has not been reported. This study aimed to characterize the dried powder of *C. aurantifolia* lime peel collected from Kediri according to Indonesian Herbal Pharmacopoeia (IHP) standards, including microbial quality parameters (Total Aerobic Plate Count/ALT and Mold-Yeast Count/AKK), and to conduct phytochemical profiling of both simplicia and the ethanolic extract. Lime peels were cleaned, dried at 40–50°C, powdered, and characterized for water content (8.64%), water-soluble extract (26.67%), ethanol-soluble extract (18.14%), total ash (5.62%), acid-insoluble ash (0.35%), ALT (3.5×10^3 CFU/g), and AKK (1.2×10^2 CFU/g)—all within IHP requirements. Phytochemical screening detected alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids in both simplicia and extract. These findings establish a standardized pharmacognostic profile supporting the use of Kediri-sourced lime peel extract in evidence-based antibacterial research.

Keywords: *Citrus aurantifolia*; ALT; AKK; lime peel; phytochemical; quality standardization

Abstrak

Citrus aurantifolia (Christm.) Swingle merupakan tanaman buah yang kulitnya sering dibuang sebagai limbah pertanian di Kediri, Jawa Timur, Indonesia, padahal berpotensi sebagai sumber senyawa bioaktif antibakteri. Standarisasi mutu bahan herbal merupakan prasyarat untuk memperoleh aktivitas farmakologis yang reprodusibel, namun karakterisasi fisikokimia dan mikrobiologi lengkap dari simplisia kulit jeruk nipis dari wilayah ini belum pernah dilaporkan. Penelitian ini bertujuan untuk mengkarakterisasi serbuk kering kulit *C. aurantifolia* asal Kediri sesuai standar Farmakope Herbal Indonesia (FHI), termasuk parameter mutu mikrobiologi (Angka Lempeng Total/ALT dan Angka Kapang Khamir/AKK), serta melakukan skrining fitokimia simplisia dan ekstrak etanol. Kulit jeruk nipis dibersihkan, dikeringkan pada 40–50°C, dihaluskan, dan dikarakterisasi: kadar air (8,64%), kadar sari larut air (26,67%), kadar sari larut etanol (18,14%), kadar abu total (5,62%), kadar abu tidak larut asam (0,35%), ALT ($3{,}5 \times 10^3$ CFU/g), dan AKK ($1{,}2 \times 10^2$ CFU/g)—seluruhnya memenuhi persyaratan FHI. Skrining fitokimia mendeteksi alkaloid, flavonoid, glikosida, saponin, tanin, dan steroid/triterpenoid pada simplisia dan ekstrak. Temuan ini memberikan profil farmakognosi terstandarisasi yang mendukung penggunaan ekstrak kulit jeruk nipis asal Kediri dalam penelitian antibakteri berbasis bukti.

Kata Kunci: *Citrus aurantifolia*; ALT; AKK; kulit jeruk nipis; fitokimia; standarisasi mutu

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INTRODUCTION

Herbal medicines have occupied a central role in traditional healthcare systems for millennia, yet their transition into contemporary clinical practice demands rigorous quality assurance frameworks. Unlike single-molecule synthetic drugs, herbal preparations are complex chemical matrices whose composition—and consequently their biological activity—varies as a function of botanical origin, geographic and pedoclimatic growing conditions, harvest timing, post-harvest processing, drying conditions, and extraction methodology.[1] This inherent variability necessitates systematic physicochemical characterization of the raw plant material (simplicia) before any pharmacological investigation is undertaken, to ensure reproducibility of results and safety of use.

Citrus aurantifolia (Christm.) Swingle (Rutaceae), commonly designated as *jeruk nipis* in Indonesian, is a tropical fruit species extensively cultivated across East Java, including the Kediri Regency, one of Indonesia's principal lime-producing regions. While the juice and pulp are commercially exploited, the peel—constituting approximately 25–30% of total fruit weight—is routinely discarded as agro-industrial waste,

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generating significant biomass with underutilised pharmacological potential.[2,3] Citrus peels are well-documented repositories of diverse phytochemicals including polymethoxylated flavones, limonoids, coumarins, alkaloids, volatile terpenoids, and phenolic acids, which collectively confer multiple bioactivities including antibacterial, antifungal, antioxidant, and anti-inflammatory properties.

Quality standardization of herbal materials in Indonesia is governed by the Farmakope Herbal Indonesia (FHI) and Peraturan Menteri Kesehatan (Permenkes) No. 007 Tahun 2012, which prescribe mandatory physicochemical and microbiological parameters for dried plant materials used in herbal pharmaceutical preparations.[4] These parameters include: (a) water content—a critical determinant of shelf stability and susceptibility to microbial spoilage; (b) water-soluble and ethanol-soluble extract contents—surrogates for the yield of pharmacologically active polar and semi-polar compounds; (c) total ash and acid-insoluble ash contents—indicators of inorganic contamination and siliceous impurities; and (d) microbiological parameters, specifically Angka Lempeng Total (ALT, Total Aerobic Plate Count) and Angka Kapang Khamir (AKK, Mold and Yeast Count)—reflecting the total viable aerobic microbial and fungal burden in the material.

Microbiological quality control of herbal materials is an often-overlooked but pharmacologically critical dimension of standardization. Elevated microbial counts in herbal simplicia pose direct risks including production of mycotoxins by contaminating fungi, bacterial toxin formation, and degradation of heat-sensitive bioactive constituents by microbial enzymes.[5] The FHI Edition II and Permenkes No. 007/2012 specify an ALT limit of ≤1 × 10^7 CFU/g and an AKK limit of ≤1 × 10^4 CFU/g for dried herbal materials. Demonstrating compliance with these limits is essential for establishing the microbiological safety of the material for pharmaceutical use.

Previous studies on *C. aurantifolia* peel have primarily reported antibacterial activity evaluations or flavonoid content analyses without addressing comprehensive standardization.[6,7] Parama et al.[2] evaluated the antibacterial efficacy of lime extract against *Streptococcus mutans* but did not characterize the raw material. Tafonao[8] investigated lime peel in deodorant formulations against *Staphylococcus epidermidis* without reporting physicochemical parameters. Meliyana and Ridwanto[9] assessed antioxidant activity of *Citrus microcarpa* leaf extract but did not address peel characterization. Notably, no published study has reported ALT and AKK data alongside the full physicochemical profile for lime peel simplicia from the Kediri growing region, creating a significant gap in the quality standardization evidence base for this material.

The geographic specificity of characterization is not trivial: studies have demonstrated that citrus peel composition varies significantly between cultivation regions due to differences in soil mineralogy, rainfall, temperature, altitude, and agricultural practice.[10] Kediri, situated in the fertile volcanic plains of East Java at elevations between 100–600 m above sea level with characteristic tropical monsoon climate, represents a distinct agroecological environment that may impart specific phytochemical and quality characteristics to locally grown lime peel.

This study therefore aimed to: (i) conduct comprehensive physicochemical characterization—including ALT and AKK microbiological parameters—of *C. aurantifolia* lime peel dried powder collected from Kediri, East Java, in full compliance with FHI Edition I/II and Permenkes No. 007/2012 standards; (ii) perform systematic qualitative phytochemical screening of both the simplicia and the 96% ethanolic extract to identify classes of secondary metabolites; and (iii) provide a validated pharmacognostic reference profile to underpin subsequent antibacterial and formulation studies using this locally sourced material.

METHODOLOGY

Research Design and Ethical Considerations

This study was designed as a descriptive-analytical laboratory investigation with a purely observational design. The research was conducted at the Laboratory of Pharmaceutical Biology, Universitas Strada Indonesia, Kediri. Botanical collection and all laboratory procedures were conducted following standard pharmacognosy protocols. No animal or human subjects were involved; thus, formal ethics committee approval was not required. All experimental procedures complied with applicable national pharmaceutical and laboratory safety regulations.

Plant Material Collection, Identification, and Preparation

Mature lime fruits (*Citrus aurantifolia* [Christm.] Swingle) were collected purposively from the Pasar Setono Betek traditional market and surrounding agricultural suppliers in Kediri City and Kediri Regency, East Java Province, Indonesia (7°49'S, 112°01'E; elevation 67 m a.s.l.) during the dry season (June–August). Botanical identification was performed by reference to authenticated specimens at the Regional Herbarium and cross-referenced against the Flora of Java (Backer and van den Brink, 1965) and monographs in the FHI. A voucher specimen was deposited at the Laboratorium Farmakognosi, Universitas Strada Indonesia (specimen no. STR-CA-2024-001).

Peels were manually separated from pulp and mesocarp, rinsed three times under running potable water, and once with distilled water to remove surface contaminants. Washed peels were drained on stainless-steel mesh racks and weighed for wet weight determination. Drying was performed in a mechanical convection oven (Memmert, Germany) at 40 ± 2°C for 72 hours or until constant weight was achieved (weight change < 0.5% between consecutive weighings). Dried peels were ground in a stainless-steel blender (Waring, USA) and sieved through a 40-mesh screen (aperture 425 μm) to yield a uniform dried powder (simplicia). The powder was stored in amber glass containers sealed with desiccant at 25°C away from direct light until analysis.[4,11]

Physicochemical Characterization of Dried Powder

All characterization parameters were determined in triplicate (n = 3) according to the methods prescribed in the Farmakope Herbal Indonesia Edition I (Depkes RI, 2008) and FHI Edition II (Kemenkes RI, 2011), and are reported as mean ± standard deviation (SD).

Water Content was determined gravimetrically using the oven-drying method. Approximately 2 g of simplicia (W_0) was placed in a pre-weighed, dried porcelain crucible and dried at 105°C to constant weight (W_1). Water content (%) = $[(W_0 - W_1) / W_0] \times 100$.

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Water-Soluble Extract Content was determined by macerating 5 g of dried powder in 100 mL distilled water for 24 hours at room temperature with intermittent stirring. The macerate was filtered through Whatman No. 41 paper; 20 mL of filtrate was evaporated to dryness at 105°C and weighed. Extract content (%) = (weight of residue / 5 g) × (100 mL/20 mL) × 100.

Ethanol-Soluble Extract Content was determined by the same procedure using 96% ethanol as solvent.

Total Ash Content was determined by incinerating 2–3 g of dried powder in a platinum crucible at 600 ± 25°C for 3 hours in a muffle furnace until light grey ash with no black particles remained. After cooling in a desiccator, the residue was weighed.

Acid-Insoluble Ash Content was determined by boiling the total ash residue with 25 mL of 2N HCl for 5 minutes, filtering through ashless filter paper (Whatman No. 41), washing with hot distilled water until neutral pH, then igniting the filter with residue at 600°C to constant weight.

Microbiological Quality Parameters (ALT and AKK)

Microbiological quality testing was performed according to Permenkes No. 007 Tahun 2012 and FHI Edition II standards, under aseptic conditions in a Class II Biological Safety Cabinet (BSC).

Angka Lempeng Total (ALT — Total Aerobic Plate Count). 1 g of dried powder was aseptically transferred into 9 mL sterile buffered peptone water (BPW) and vortexed for 1 minute to yield a 10^{-1} dilution. Serial decimal dilutions (10^{-2} to 10^{-5}) were prepared in BPW. From each dilution, 1 mL aliquots were pour-plated in duplicate onto sterile Tryptone Soy Agar (TSA; Himedia). Plates were incubated at 37 ± 1°C for 48 ± 3 hours. Colonies were counted using a digital colony counter (Stuart, UK) and results expressed as CFU/g. Plates with 25–250 colonies were used for calculation. The regulatory limit for dried herbal simplicia is ≤1 × 10^7 CFU/g.[4,5]

Angka Kapang Khamir (AKK — Mold and Yeast Count). Using the same dilution series, 1 mL aliquots from each dilution were pour-plated in duplicate onto Potato Dextrose Agar (PDA; Himedia) supplemented with chloramphenicol (0.1 g/L) to inhibit bacterial growth. Plates were incubated at 25 ± 1°C for 120 ± 24 hours (5 days). Fungal colonies (molds identified by macroscopic morphology and aerial hyphal growth; yeasts as smooth, opaque colonies) were counted and results expressed as CFU/g. The regulatory limit is ≤1 × 10^4 CFU/g.[4,5]

Preparation of Ethanolic Extract

Five hundred grams (500 g) of dried lime peel powder was macerated in a sealed 5-L amber glass vessel with 3,750 mL (75 parts) of 96% pharmaceutical-grade ethanol (pa) at room temperature (25 ± 2°C) for 5 days with stirring every 24 hours. The macerate was filtered through a Buchner funnel with Whatman No. 41 paper. The residue was re-macerated with the remaining 1,250 mL (25 parts) of 96% ethanol for 2 additional days.[11] The combined filtrate was concentrated using a rotary vacuum evaporator (Buchi R-300, Switzerland) at 40°C, 150 rpm, under reduced pressure (200 mbar) until a semi-solid viscous extract was obtained. Extraction yield (% w/w) was calculated relative to the initial dried powder weight. The extract was stored in sealed amber glass vials at 4°C until use.

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Phytochemical Screening

Qualitative phytochemical screening was performed on both the dried powder (simplicia) and the ethanolic extract following the methods of Farnsworth[12] and Harborne[13] for six compound classes:

Alkaloids were detected using three precipitant reagents: Dragendorff (potassium bismuth iodide—orange-red precipitate positive), Bouchardat (iodine-potassium iodide—brown/dark precipitate positive), and Mayer (potassium mercuric iodide—cream/white precipitate positive). A positive result required ≥2 of 3 reagents to precipitate.

Flavonoids were identified by the Shinoda test: sample dissolved in 96% ethanol was treated with magnesium ribbon (Mg) and 5 drops of concentrated HCl; the mixture was shaken with 2 mL amyl alcohol. Red-orange to red colouration of the amyl alcohol layer indicated a positive result.

Glycosides were detected using the Molisch test: 1 mL of aqueous sample extract was treated with 2 drops of 10% alpha-naphthol solution in ethanol, then 2 mL concentrated H_2SO_4 was carefully added along the tube wall. Formation of a purple-violet ring at the liquid interface indicated a positive result.

Saponins were identified by the foam stability test: 0.5 g of sample was transferred to a graduated test tube with 10 mL hot distilled water and vigorously shaken vertically for 30 seconds. Persistent foam height >1 cm after standing for 10 minutes indicated a positive result.

Tannins were detected by treating 2 mL of aqueous extract with 3 drops of 1% ferric chloride ($FeCl_3$) solution. Formation of a blue-black (hydrolysable tannins) or blue-green (condensed tannins) colour indicated a positive result.

Steroids and Triterpenoids were identified using the Liebermann-Burchard test: 2 mL of extract dissolved in chloroform was treated with 10 drops of acetic anhydride and 2 drops of concentrated H_2SO_4. A blue-green colour indicated steroids; a red-purple colour indicated triterpenoids.

Data Analysis

All characterization data are expressed as mean ± standard deviation (SD) of triplicate determinations (n = 3). Results were compared against IHP Edition I/II and Permenkes No. 007/2012 requirements. Descriptive statistical analysis was performed using Microsoft Excel 2019.

RESULTS AND DISCUSSION

Macroscopic Examination and Extraction Yield

Macroscopic evaluation of the lime peel dried powder revealed a pale yellow-green coloration, characteristic citrus aroma attributable to volatile limonene and linalool, and a moderately rough texture. Gross morphological features were consistent with authenticated *Citrus aurantifolia* peel material as described in the Materia Medika Indonesia and standard herbal monographs. The wet-to-dry weight ratio was approximately 4.5:1, indicating a 78% moisture loss during drying, consistent with reported values for citrus peels.[9] Extraction of 500 g dried powder by ethanol maceration yielded 84.3 g of thick semi-solid extract, corresponding to an extraction yield of 16.86% (w/w). This yield is within the range reported for *Citrus* peel extracts (12–22% w/w depending on solvent polarity and extraction parameters).[7]

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Physicochemical and Microbiological Characterization

The complete characterization profile of lime peel dried powder, including both physicochemical parameters and microbiological quality indicators (ALT and AKK), is presented in Table 1. All seven parameters evaluated were within the limits specified by the Farmakope Herbal Indonesia (FHI) Edition I (Depkes RI, 2008), FHI Edition II (Kemenkes RI, 2011), and Permenkes No. 007 Tahun 2012, confirming full compliance with national herbal quality standards.

Table 1. Physicochemical and microbiological characterization of *Citrus aurantifolia* lime peel dried powder from Kediri

No.	Parameter	Result (Mean ± SD)	IHP / Permenkes No.007/2012 Requirement
1.	Water Content	8.64 ± 0.12%	≤ 10%
2.	Water-Soluble Extract Content	26.67 ± 0.43%	≥ 4.4%
3.	Ethanol-Soluble Extract Content	18.14 ± 0.38%	≥ 15.4%
4.	Total Ash Content	5.62 ± 0.09%	≤ 16.6%
5.	Acid-Insoluble Ash Content	0.35 ± 0.02%	≤ 0.7%
6.	ALT (Total Aerobic Plate Count)	3.5×10^3 CFU/g	$\leq 1 \times 10^7$ CFU/g
7.	AKK (Mold and Yeast Count)	1.2×10^2 CFU/g	$\leq 1 \times 10^4$ CFU/g

ALT: Angka Lempeng Total; AKK: Angka Kapang Khamir; SD: standard deviation of triplicate determinations (n = 3). Requirements per FHI Edition I/II and Permenkes No. 007/2012.

Water Content. The measured water content of 8.64 ± 0.12% is substantially below the FHI maximum of 10%, confirming the effectiveness of the drying protocol. Water activity is the primary determinant of microbial growth and enzymatic degradation in dried plant materials; water content above 10% exponentially increases the risk of hydrolytic enzyme activity and secondary fungal contamination, particularly by *Aspergillus* and *Penicillium* species that produce hepatotoxic aflatoxins.[5,14] The drying temperature of 40–50°C was deliberately selected to remain below the thermal degradation threshold of thermolabile phenolic glycosides and flavonoids in citrus peel (typically >60°C), while achieving sufficient moisture reduction. The result is consistent with values reported for lime peel from other Indonesian production regions (7.8–9.1%).[9]

Soluble Extract Contents. The water-soluble extract content (26.67 ± 0.43%) substantially exceeds the FHI minimum of 4.4%, indicating a high content of polar compounds—primarily flavonoid glycosides (naringin, hesperidin, rutin), phenolic acids (chlorogenic acid, caffeic acid), and water-soluble saponins.[6] The ethanol-soluble extract content (18.14 ± 0.38%), while lower than the water-soluble fraction, still comfortably exceeds the FHI minimum of 15.4%. The higher water solubility relative to ethanol solubility (ratio 1.47:1) indicates a predominantly polar phytochemical composition in this material, consistent with the pattern reported for *Citrus* spp. peels processed under similar conditions.[6,10] This polar-dominant profile has favourable implications for aqueous or hydroethanolic extraction in pharmaceutical formulation development.

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Ash Contents. Total ash content of 5.62 ± 0.09% represents the total inorganic residue from intrinsic physiological minerals (Ca, K, Mg, P) and any extrinsic soil/dust contamination. The measured value is well below the FHI maximum (16.6%), indicating clean, well-washed material with low extraneous mineral contamination. Acid-insoluble ash (0.35 ± 0.02%), representing primarily siliceous matter (SiO_2) that is insoluble in hydrochloric acid and indicative of sand/soil contamination, is also far below the FHI limit of 0.7%.[15] Both ash parameters confirm the adequacy of the collection and washing procedures for the Kediri-sourced material.

Angka Lempeng Total (ALT). The ALT of 3.5×10^3 CFU/g is three orders of magnitude below the regulatory limit of 1×10^7 CFU/g specified in Permenkes No. 007/2012 for dried herbal materials.[4] This low microbial count confirms that the triple-washing step (twice with potable water, once with distilled water) combined with convection oven drying at 40°C effectively reduced surface microbial contamination to pharmacologically safe levels. Comparative literature data for undried citrus peel report ALT values of 10^5–10^6 CFU/g, indicating that the drying process achieved approximately 2–3 $\log_{10}$ CFU/g reduction in viable aerobic bacteria.[5] The low ALT value also confirms that microbial enzymatic degradation of bioactive secondary metabolites is unlikely during storage under the specified conditions.

Angka Kapang Khamir (AKK). The AKK of 1.2×10^2 CFU/g is two orders of magnitude below the regulatory limit of 1×10^4 CFU/g.[4] Fungi represent the primary spoilage organisms in dried plant materials due to their capacity to grow at water activities (a_w) as low as 0.70–0.75 and to produce mycotoxins (aflatoxins, ochratoxins, fumonisins) that are toxic to humans even at microgram concentrations.[14] The very low AKK (1.2×10^2 CFU/g) obtained in this study strongly supports the safety of the material for pharmaceutical applications and reflects both the effectiveness of the drying process and the appropriate storage conditions employed. To our knowledge, this is the first report of ALT and AKK values for lime peel simplicia specifically sourced from the Kediri region, filling an important gap in the regional quality standardization literature.

Phytochemical Screening

The results of qualitative phytochemical screening of both the dried powder (simplicia) and the 96% ethanolic extract are presented in Table 2. All six compound classes were detected in both forms of the material, confirming the ethanolic maceration process effectively extracted the representative chemical classes present in the simplicia without selective exclusion.

Table 2. Phytochemical screening of *C. aurantifolia* lime peel simplicia and ethanolic extract

No.	Secondary Metabolite	Reagent(s)	Simplicia	Ethanolic Extract
1.	Alkaloid	Dragendorff; Bouchardat; Mayer	+	+
2.	Flavonoid	Mg ribbon + conc. HCl + amyl alcohol	+	+
3.	Glycoside	Molisch + H_2SO_4 (conc.)	+	+
4.	Saponin	Foam stability test (hot water)	+	+
5.	Tannin	1% $FeCl_3$ solution	+	+
6.	Steroid / Triterpenoid	Liebermann-Burchard	+	+

(+): compound class detected; (−): not detected. Triplicate testing performed for each compound class.

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Alkaloids. All three precipitant reagents (Dragendorff, Bouchardat, and Mayer) produced positive reactions in both simplicia and extract. The Rutaceae family is characterised by acridone alkaloids, quinoline alkaloids, and furoquinoline alkaloids—compounds specifically biosynthesised in *Citrus* spp. via the anthranilate pathway.[6,16] Acridone alkaloids such as acronycine and noracronycine in citrus peel possess documented antibacterial activity by intercalating into bacterial DNA and inhibiting DNA gyrase, consistent with the antibacterial properties observed in subsequent activity studies.

Flavonoids. The characteristic red-orange colour development in the amyl alcohol layer (Shinoda test) confirmed the presence of flavonoids in both simplicia and extract. *Citrus aurantifolia* peel is a rich source of flavanones—primarily naringenin, hesperetin, and eriocitrin—as well as polymethoxylated flavones (PMFs) such as nobiletin and tangeretin, which are essentially unique to *Citrus* among higher plants.[6] These compounds are concentrated in the flavedo (outer coloured layer) of the peel and are the primary contributors to antibacterial, antioxidant, and anti-inflammatory activity. Their amphipathic structure allows intercalation into bacterial phospholipid bilayers and inhibition of peptidoglycan transpeptidation.

Glycosides. The positive Molisch reaction indicates the presence of carbohydrate moieties, most likely in the form of flavonoid glycosides (flavanone-7-O-rutinosides, flavanone-7-O-neohesperidosides) and terpenoid glycosides characteristic of citrus peel.[13] Glycosylation enhances aqueous solubility of the parent aglycone, facilitating extraction in aqueous systems and bioavailability in polar biological environments.

Saponins. The formation of persistent, stable foam (>1 cm for >10 minutes) in both forms confirmed the presence of saponins. Citrus peels contain triterpenoid saponins (limonoids and their glycosides, notably limonin and nomilin glucosides).[17] Saponins disrupt bacterial cell membranes through an amphipathic interaction with membrane phospholipids and cholesterol, forming pores that cause leakage of intracellular contents—a mechanism distinct from, and synergistic with, flavonoid and alkaloid antibacterial actions.

Tannins. The intense blue-green coloration with $FeCl_3$ indicates the predominant presence of condensed tannins (proanthocyanidins), which are polymerised flavan-3-ol units resistant to hydrolysis.[12] Tannins exert antibacterial activity through multiple mechanisms: (1) complex formation with bacterial surface proteins, inhibiting adhesion and colonisation; (2) chelation of divalent metal ions (Fe^{2+}, Mg^{2+}, Zn^{2+}) essential as enzyme cofactors; (3) inhibition of extracellular enzymes (lipases, proteases, hyaluronidases) secreted by pathogenic bacteria such as *Propionibacterium acnes* and *Staphylococcus epidermidis*.

Steroids/Triterpenoids. The Liebermann-Burchard test produced a blue-green colour indicative of sterols and a faint red-purple tinge suggesting triterpenoids. *Citrus* spp. peels are known to contain β-sitosterol, stigmasterol, and campesterol (phytosterols) alongside the limonoid triterpenoids limonin, nomilin, and obacunone.[17] Limonoids demonstrate biological activity including antibacterial, antitumour, and insecticidal properties, and contribute to the characteristic bitterness of

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lime peel extract. The detection of steroids/triterpenoids in both forms confirms the efficacy of ethanol in co-extracting these relatively apolar compounds alongside the more polar glycosides and tannins.

The comprehensive phytochemical profile detected—alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids—in the Kediri-sourced lime peel is consistent with published reports for *Citrus aurantifolia* from other Indonesian and Southeast Asian production regions.[6,7,8] The concordance between simplicia and extract profiles confirms that the 5+2-day ethanol maceration protocol effectively solubilises the full spectrum of bioactive compound classes, making the extract a pharmacologically representative preparation of the simplicia. This is pharmacognostically significant because it validates the direct extrapolation of simplicia-level quality parameters to the extract used in downstream antibacterial studies.

The multi-compound profile identified here has important pharmacological implications. Antibacterial activity of plant extracts rarely derives from a single compound; rather, it reflects synergistic interactions among multiple bioactive constituents acting on different bacterial targets simultaneously—a multi-target mechanism that is fundamentally distinct from single-molecule antibiotics and offers the theoretical advantage of reduced resistance development probability.[16,18] For lime peel extract, the alkaloids, flavonoids, tannins, and saponins each contribute distinct antibacterial mechanisms (DNA intercalation/gyrase inhibition, cell wall transpeptidation inhibition, surface protein precipitation, and membrane pore formation, respectively), creating an intrinsically multi-target antibacterial profile.

CONCLUSION

Citrus aurantifolia lime peel simplicia sourced from Kediri, East Java, demonstrated full compliance with Farmakope Herbal Indonesia Edition I/II and Permenkes No. 007/2012 standards across all evaluated physicochemical and microbiological parameters, confirming its purity, stability, and safety for pharmaceutical application. Phytochemical screening consistently identified alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids in both simplicia and ethanolic extract, indicating that the maceration process effectively co-extracted representative bioactive compound classes. These findings establish a validated pharmacognostic reference profile for Kediri-derived lime peel, providing a standardized quality foundation for subsequent antibacterial and formulation research. Future investigations should prioritize quantitative marker analysis, stability assessment, and mechanistic antibacterial studies to advance evidence-based pharmaceutical development.

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