Comparative Antibacterial Activity of Beluntas (Pluchea indica L.) Leaf Ethanolic Extracts Obtained by Maceration and Ultrasound-Assisted Extraction Against Three Acne-Associated Bacteria: An In Vitro Study

Ernoviya[1]*, Zulfikri[1]

[1]Department of Pharmacy, Politeknik Kesehatan Kemenkes Medan, Medan, North Sumatra, Indonesia

*Email correspondence: ernoviya73@gmail.com

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Abstract

Rising resistance of acne-associated Gram-positive bacteria, including Cutibacterium acnes (formerly Propionibacterium acnes), Staphylococcus epidermidis, and Staphylococcus aureus, to first-line macrolide and tetracycline antibiotics has become a global dermatological and public-health concern, intensifying the search for plant-derived antibacterial alternatives. Beluntas (Pluchea indica L., Asteraceae) is widely used in Indonesian traditional medicine and contains flavonoids, tannins, saponins, and caffeoylquinic acids; however, the influence of extraction technique on its antibacterial potency against acne-associated bacteria has not been systematically evaluated. This study aimed to compare the in vitro antibacterial activity of P. indica leaf ethanolic extracts prepared by 5-day cold maceration and 60-minute ultrasound-assisted extraction (UAE, <20 kHz) against C. acnes, S. epidermidis and S. aureus. Powdered leaves were extracted with 70% ethanol using either 5-day cold maceration or 60-min UAE. Following concentration with a rotary evaporator, six concentrations (10–60%, w/v) were evaluated using the agar well diffusion method on Mueller–Hinton agar for S. aureus, S. epidermidis and blood agar for C. acnes. Inhibition-zone diameters were interpreted according to the Davis–Stout classification. Phytochemical screening confirmed the presence of flavonoids, triterpenoids, glycosides, saponins and tannins. Maceration produced larger inhibition zones than UAE for all three organisms at every concentration tested (single-measurement values). The largest inhibition was observed against S. aureus with the 60% maceration extract (20.30 mm; very strong category) followed by S. epidermidis (14.70 mm; strong) and C. acnes (13.65 mm; strong). The susceptibility ranking was S. aureus > S. epidermidis > C. acnes. P. Ethanolic leaf extracts of P. indica obtained through maceration exhibited stronger antibacterial activity than those through UAE against acne-associated Gram-positive bacteria. These preliminary findings suggest that beluntas leaf extract is a promising plant-based antibacterial candidate that may help reduce reliance on synthetic antibiotics and support its further development as a complementary or preventive anti-acne ingredient, pending confirmatory studies incorporating appropriate replication and controls.

Keywords: Acne Vulgaris, Antimicrobial Resistance, Dermatological Phytotherapy, Maceration, Pluchea Indica, Ultrasound-Assisted Extraction.

INTRODUCTION

Antimicrobial resistance (AMR) has become one of the most significant threats to global public health (Naghavi et al., 2024). The Global Burden of Disease Consortium estimated that 4.71 million deaths were associated with bacterial AMR in 2021, and projected more than 39 million cumulative AMR-attributable deaths between 2025 and 2050 if current trends continue (Naghavi et al., 2024). Skin and soft-tissue infections are among the conditions most affected by this trend, with *Staphylococcus aureus* alone accounting for an estimated 226 cases per 100,000 population annually in high-resource settings (Phillip et al., 2023).

Acne vulgaris affects up to 9.4% of the global population and approximately 80% of adolescents during puberty (Mohammadi et al., 2024; Rouvier et al., 2024). The condition involves a polymicrobial process in which *Cutibacterium acnes*, a Gram-positive anaerobic-to-aerotolerant bacterium reclassified from *Propionibacterium acnes* in 2016, proliferates within sebum-rich pilosebaceous units. This process is accompanied by *Staphylococcus epidermidis,* which contributes to biofilm formation. and *S. aureus,* which is frequently associated with inflammatory lesions (Sun et al., 2024).

Topical and oral antibiotics, particularly macrolides and tetracyclines, remain first-line treatments for inflammatory acne; however, antimicrobial resistance has increased substantially. A systematic review and meta-analysis spanning four decades and 27 countries reported that clindamycin resistance in *C. acnes* increased from 25.5% during 1983–2014 to 35.4% during 2015–2023, while erythromycin resistance exceeded 50% in several regions (Beig et al., 2024). Among Egyptian patients with acne vulgaris, resistance rates for erythromycin, clindamycin, and tetracycline reached 70% or higher (Khashaba et al., 2024). Furthermore, a recent meta-analysis covering the period from 2005 to 2025 confirmed the continued global escalation of resistance to both macrolide and tetracycline (Zhu et al., 2025).

These trends have intensified the search for naturally derived antibacterial agents (Sun et al., 2024). *Pluchea indica* (L.) Less. (Asteraceae), locally known as *beluntas*, is a coastal shrub widely consumed and used in traditional medicine throughout Indonesia and Southeast Asia for treating body odour, dyspepsia, and skin conditions (Chan et al., 2022; Ibrahim et al., 2022). Recent reviews have documented its anti-infective potential against a broad spectrum of Gram-positive and Gram-negative pathogens, including *S. aureus*, *S. epidermidis* and *C. acnes* (Hikmawanti et al., 2024). Notably, although the ethnobotanical and phytochemical literature on *P. indica* is extensive, direct comparisons between conventional extraction methods (maceration) and modern techniques (ultrasound-assisted extraction) specifically targeting acne-causing pathogens remain scarce. This gap in the literature provides a clear rationale for the present study addresses.

The antibacterial activity of *P. indica* is attributed to its rich content of secondary-metabolite, including flavonoids, tannins, saponins, alkaloids, triterpenoids, and caffeoylquinic acids. Among these, 4,5-O-dicaffeoylquinic acid has been identified as a major bioactive compound (Chiangnoon et al., 2022; Donowarti et al., 2024). These constituents are believed to exert antibacterial effects through complementary and potentially synergistic mechanisms. Flavonoids can disrupt bacterial cell wall and membrane integrity and inhibit the NorA efflux pump in *S. aureus* (Veiko et al., 2023; Zhou et al., 2023). Tannins precipitate bacterial surface

proteins and impair biofilm formation in both methicillin-sensitive and methicillin-resistant *S. aureus* (Qin et al., 2023). Whereas saponins compromise membrane lipid bilayers in Gram-positive bacteria (Kheirandish et al., 2024). Collectively, these mechanisms, including membrane disruption, protein precipitation, efflux-pump inhibition, and interference with biofilm formation, may compromise bacterial cell-envelope integrity more effectively than any single class of compounds alone. However, such synergistic activity has not yet been experimentally confirmed for *P. indica*.

The extraction technique influences not only the yield but also the chemical composition and biological activity of plant extracts (Hao et al., 2023). Maceration is a simple, low-temperature solid–liquid extraction method that preserves heat-labile metabolites; however, is relatively time-consuming. Ultrasound-assisted extraction (UAE), in which acoustic cavitation enhances solvent penetration into plant cells, has been proposed as a faster and more environmentally friendly alternative and often produces higher yields of flavonoids and polyphenols (Hao et al., 2023; Mossie et al., 2024). However, the outcomes are not always consistent. UAE may promote the thermal or oxidative degradation of certain polyphenolic compounds, resulting in lower biological activity than that achieved with conventional maceration in some plant matrices (Mossie et al., 2024).

For *P. indica* specifically, direct comparative data on the antibacterial activity of extracts obtained through maceration and UAE against acne-associated bacteria remain limited. Therefore, the present study aimed to compare the *in vitro* antibacterial activity of *P. indica* leaf ethanolic extracts prepared by maceration and UAE against *C. acnes, S. epidermidis* and *S. aureus* at six concentrations (10–60% w/v), using the agar well diffusion method. We hypothesised that, despite the generally higher flavonoid and polyphenol yields reported for UAE due to cavitation-driven cell-wall disruption, the shorter extraction time may not fully offset the potential effects of localised thermal and oxidative stress. Consequently, conventional cold maceration was expected to preserve heat-labile caffeoylquinic acid derivatives more effectively and exhibit greater antibacterial activity than UAE.

METHODS

Study design and setting

This in vitro experimental study was conducted at the Pharmacy Laboratory of Politeknik Kesehatan Kemenkes Medan and the Pharmacy Laboratory of Universitas Sumatera Utara, Medan, North Sumatra, Indonesia, between January and October 2025.

Plant material

Fresh leaves of *P. indica* were collected, cleaned, air-dried at ambient temperature away from direct sunlight, milled, and sieved through a 100-mesh sieve. The resulting powder was stored in sealed containers protected from light until use.

Extraction by maceration

Following the Farmakope Herbal Indonesia (2017) protocol (Kemenkes RI, 2017), 200 g of leaf powder was macerated with 1,845.24 mL of 70% ethanol in a sealed container at room temperature for 5 days under light-protected conditions with intermittent stirring. The macerate was filtered, and the residue re-extracted with an additional 615.08 mL of 70% ethanol for 2 days. The combined filtrates were concentrated using a rotary vacuum evaporator at 40 °C until a viscous extract was obtained.

A 70% ethanol concentration was selected on the *Farmakope Herbal Indonesia* protocol because it provides sufficient polarity to solubilise phenolics, flavonoids, and saponins from *P. indica* leaves while limiting the co-extraction of non-polar, pharmacologically inert constituents (Kemenkes RI, 2017). The test concentration range (10–60% w/v) was predetermined to characterize the full dose-response relationship of theextract without exceeding the practical diffusion capacity of the agar well diffusion assay.

Ultrasound-assisted extraction

For ultrasound-assisted extraction (UAE), 200 g of leaf powder was suspended in 2,460.32 mL of 70% ethanol and sonicated in an ultrasonic bath at <20 kHz for 60 min at room temperature. The filtrate was concentrated under the same conditions used for the maceration extract.

Phytochemical screening

Standard colour-reaction tests were performed on both extracts to identify alkaloids (Bouchardat, Meyer, and Dragendorff reagents), flavonoids (Mg–HCl with H_2SO_4), triterpenoids and steroids (Liebermann–Burchard), glycosides (Molisch with H_2SO_4), saponins (foam-formation test), and tannins (1% $FeCl_3$). This is one of the qualitative tests performed to identify active compounds present in *Pluchea indica L.* (*beluntas*) leaves. The classes of active compounds, or secondary metabolites, tested include phenolics, flavonoids, alkaloids, saponins, terpenoids, and steroids. A positive result for flavonoids is indicated by the formation of an orange or yellow colour. Flavonoids are inherently polar compounds; consequently, they dissolve readily in polar solvents, consistent with the principle that a compound dissolves easily in a solvent of similar polarity.

Test bacteria and inoculum preparation

The reference strains used were *Cutibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus.* The official ATCC strain numbers for the three bacteria are *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, and *Propionibacterium acnes* ATCC 11827. Bacterial suspensions were standardised to the 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL) prior to inoculation.

Antibacterial assay

Antibacterial activity was determined by the agar well diffusion method. Mueller–Hinton agar (MHA) was used for *S. aureus* and *S. epidermidis*; blood agar was used for the anaerobe *C. acnes*. Plates were inoculated by streaking the standardised bacterial suspension uniformly across the agar surface using a sterile swab. Wells of 6 mm diameter were cut with a sterile cork-borer. Test extracts at 10%, 20%, 30%, 40%, 50% and 60% w/v in carboxymethylcellulose (CMC) vehicle were dispensed (50 µL per well) using a sterile micropipette inside a laminar-flow cabinet. Plates inoculated with *C. acnes* were incubated anaerobically at 37 °C for 24 h; plates with the aerobic strains were incubated under standard atmosphere at 37 °C for 24 h. Inhibition-zone diameters were measured in millimetres using a digital caliper and were interpreted using the Davis–Stout criteria as weak (<5 mm), moderate (5–10 mm), strong (10–20 mm) or very strong (>20 mm).

Methodological transparency

Given the exploratory nature of this preliminary study, formal positive controls (clindamycin or tetracycline antibiotic discs) and a vehicle-only negative control (CMC) were not included. Inhibition zones were measured once per well without replication, and no inferential statistical analyses were performed. These methodological limitations are acknowledged and discussed in the *Limitations* subsection of the Discussion.

RESULTS

Phytochemical screening

The 70% ethanolic extract of *P. indica* leaves produced positive reactions for flavonoids, triterpenoids/steroids, glycosides, saponins and tannins; alkaloids tested negative under the conditions used (Table 1).

Table 1. Phytochemical screening of the 70% ethanolic extract of *P. indica* leaves

Phytochemical test	Reagent	Result
Alkaloids	Bouchardat, Meyer, Dragendorff	–
Flavonoids	Mg–HCl + H_2SO_4	+
Triterpenoids / steroids	Liebermann–Burchard	+
Glycosides	Molisch + H_2SO_4	+
Saponins	Foam test (aquadest)	+
Tannins	$FeCl_3$ 1%	+

Note: (+) positive — secondary metabolite detected; (–) negative — secondary metabolite not detected under the conditions used.

Antibacterial activity

Inhibition-zone diameters produced by both extraction methods across the six tested concentrations are summarised in Table 2 and Figure 1. The 6 mm baseline reflects the well diameter and is reported where no surrounding zone of growth inhibition was observed. For all three organisms, maceration extracts produced larger inhibition zones than UAE extracts at every concentration tested, and the inhibition zone increased with concentration; because each value in Table 2 represents a single measurement rather than a replicate mean, this pattern should be read as a consistent directional trend rather than a statistically confirmed difference (see Limitations).

Against *C. acnes*, the maceration extract produced inhibition zones ranging from 6.00 mm at 10% to 13.65 mm at 60%, whereas the UAE extract produced 6.00–10.90 mm over the same range. Against *S. epidermidis*, maceration zones rose from 6.00 to 14.70 mm and UAE zones from 6.00 to 9.70 mm. The strongest activity was recorded against *S. aureus*: maceration produced 11.15 mm at 10% and 20.30 mm at 60% (single measurement), the latter falling within the very strong category under the Davis–Stout criteria; UAE produced 6.00–10.90 mm. Overall susceptibility ranking was *S. aureus* > *S. epidermidis* > *C. acnes*.

Notably, UAE extracts produced no measurable zone beyond the 6 mm well at the two lowest concentrations (10–20% w/v) for all three organisms except a marginal zone against *C. acnes* at 30%. This threshold-like pattern is consistent with an extraction-efficiency limitation: at low w/v concentrations, the absolute quantity of active caffeoylquinic acid derivatives and flavonoids delivered per well may fall below the minimum local concentration required to produce a visible zone, an effect that could be compounded if sonication-induced thermal or oxidative stress had already partially degraded these labile compounds during processing (see Discussion).

Table 2. Antibacterial activity (inhibition-zone diameter, mm) of *P. indica* leaf ethanolic extract obtained by maceration versus ultrasound-assisted extraction (UAE) against three acne-associated bacteria

Method	Conc. (% w/v)	C. acnes (mm)	S. epidermidis (mm)	S. aureus (mm)
Maceration	10	6.00	6.00	11.15
	20	9.20	6.00	14.30
	30	10.50	10.00	16.95
	40	11.45	12.10	17.20
	50	11.70	13.50	18.70
	60	13.65	14.70	20.30
UAE	10	6.00	6.00	6.00
	20	6.00	6.00	6.00
	30	6.95	6.00	6.00
	40	8.15	6.00	8.80
	50	10.70	7.00	10.30
	60	10.90	9.70	10.90

Note: Values represent inhibition-zone diameter in mm including the 6-mm well, from a single measurement per well (n = 1); values of 6.00 mm therefore indicate no measurable inhibition beyond the well. The 60-min UAE frequency is stated as <20 kHz pending author verification (see Methods note); maceration was performed for 5 days at room temperature. Categorisation (Davis–Stout): <5 mm weak, 5–10 mm moderate, 10–20 mm strong, >20 mm very strong.

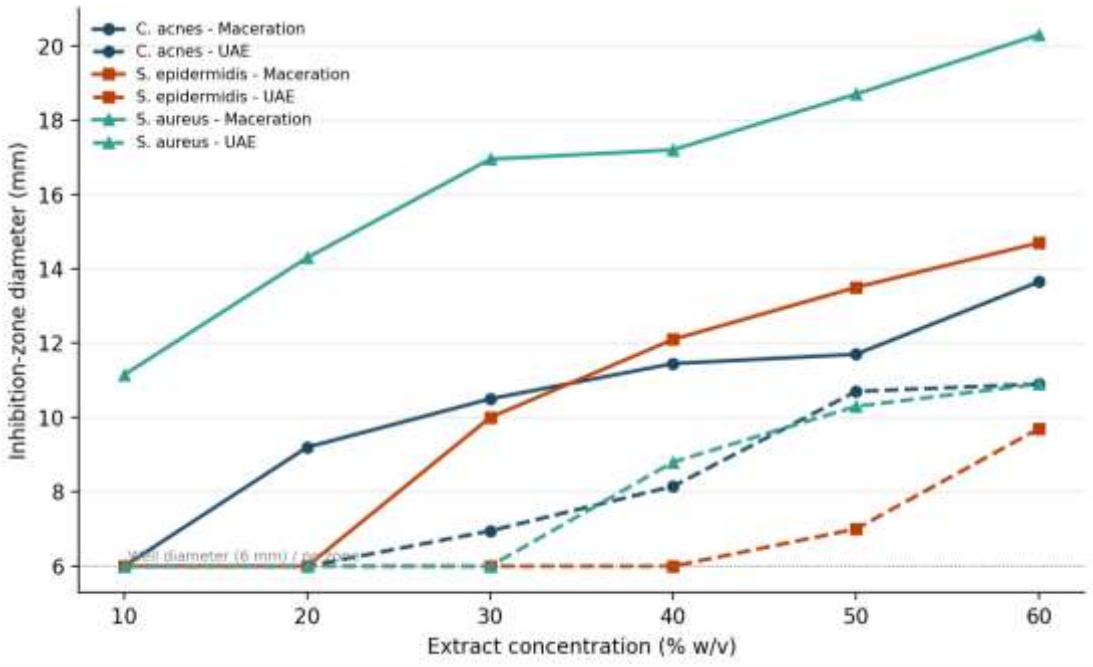

Figure 1. Dose–response comparison of inhibition-zone diameter between maceration and ultrasound-assisted extraction (UAE) against *C. acnes*, *S. epidermidis* and *S. aureus* across six extract concentrations (10–60% w/v). Solid lines: maceration; dashed lines: UAE. The horizontal dotted line marks the 6 mm well diameter (no measurable inhibition).

DISCUSSION

This in vitro comparative study demonstrated that 70% ethanolic extracts of *P. indica* leaves prepared by maceration consistently produced larger inhibition zones than those prepared by UAE against *C. acnes*, *S. epidermidis* and *S. aureus*, with the strongest single-measurement activity recorded against *S. aureus* at 60% concentration (20.30 mm). Inhibition

increased with concentration, indicating a dose–response relationship, and the overall susceptibility ranking was *S. aureus* > *S. epidermidis* > *C. acnes*.

The susceptibility ranking observed here mirrors the pattern documented by Wahyuni et al., (2024), demonstrating the ethanolic extract of *P. indica* leaves inhibited multiple Gram-positive strains in vitro, with molecular-docking analyses implicating quercetin and apigenin as key active ligands. Earlier work using *P. indica* against other Gram-positive organisms ,*Enterococcus faecalis* and *Fusobacterium nucleatum* , reported strong to moderate inhibition at concentrations of 12.5–100% (Pargaputri et al., 2016). More recent work confirmed inhibition of *S. aureus*-associated biofilm formation by *P. indica* ethanol extract exceeding 55% at higher concentrations (Yahya et al., 2025), broadly supporting our findings. The antibacterial versatility of *beluntas* leaf extract has also been documented against other bacterial targets and in other delivery formats: ethanolic extract has shown activity against *Streptococcus sanguinis* (Adiwijaya et al., 2023), fermentation with *Saccharomyces cerevisiae* has been reported to further enhance its antibacterial potency (Rismawati et al., 2022), and purified *beluntas* leaf extract has been successfully formulated into an edible anti-stomatitis film (Pratiwi & Tobi, 2023), together illustrating the plant's versatility across bacterial targets and formulation strategies.

It should be emphasised that *C. acnes* and *S. aureus* are both Gram-positive organisms (Sun et al., 2024). Therefore, the differential susceptibility observed in this study cannot be explained by Gram status alone. *S. aureus* possesses a thick peptidoglycan layer but lacks the outer lipopolysaccharide membrane characteristic of Gram-negative bacteria, allowing polar polyphenols and flavonoids in the extract to access the cytoplasmic membrane relatively easily (Veiko et al., 2023). In contrast, *C. acnes*, although also Gram-positive, inhabits lipid-rich, oxygen-poor pilosebaceous units and grows under anaerobic-to-aerotolerant conditions. These environmental and membrane composition differences may reduce penetration of polar extract constituents compared with the more accessible cell envelope of *S. aureus*. The intermediate susceptibility of *S. epidermidis* is consistent with its well-documented ability to form biofilms, which can limit the diffusion of antibacterial agents throughout the microbial population (Sun et al., 2024).

Mechanistically, flavonoids in *P. indica* have been reported to disrupt bacterial membrane integrity, inhibit enzymes essential for peptidoglycan biosynthesis, and suppress efflux pumps such as NorA in *S. aureus*, thereby potentially increasing intracellular drug accumulation (Veiko et al., 2023; Zhou et al., 2023). Tannins have been reported to precipitate bacterial surface proteins and chelate metal ions required for respiration. In both MSSA and

MRSA, tannins may also inhibit biofilm formation by interfering with the *icaA*, *agrA* and *sarA* quorum-sensing genes (Qin et al., 2023). Saponins have likewise reported to disrupt membrane lipid bilayers and dissipate membrane potential (Kheirandish et al., 2024). At the structural level, recent structure–activity reviews indicate that the antibacterial potency of flavonoids is highly dependent on hydroxylation patterns and other structural modifications (Zhang et al., 2024), which may help explain differences in bioactivity among extraction methods. It should be emphasized, however, that these mechanistic explanations are derived from studies of isolated compounds or the extracts evaluated in the present study. Thus, they should be regarded as plausible working hypotheses for the observed antibacterial activity rather than mechanisms confirmed by the present data.

Although UAE is widely reported to enhance flavonoid and polyphenol yields through cavitation-driven cell-wall disruption (Hao et al., 2023), our findings demonstrated greater antibacterial activity in maceration extracts. Several factors may account for this observation. First, prolonged cold maceration allows gradual solvent penetration and saturation of plant tissues without thermal stress, thereby preserving heat-labile caffeoylquinic acids and certain flavonols (Mossie et al., 2024). Second, 60-minute sonication can generate localised hot-spots and free radicals through acoustic cavitation.

This phenomenon has been implicated in the degradation of labile phenolic compounds, including caffeoylquinic acid derivatives, potentially reducing overall bioactivity even when total extractable polyphenol content is increased (Mossie et al., 2024). Third, the slightly different solvent-to-solid ratios used in the two protocols (1:9.2 for maceration vs. 1:12.3 for UAE, v/w) represent a potential confounding factor that may have independently influenced extraction efficiency. Consequently, the comparison should be interpreted as an evaluation of extraction-protocols rather than a fully controlled assessment of extraction technique alone. These hypotheses should be examined in future studies through quantitative determination of total phenolic content (Folin–Ciocalteu) and total flavonoid content ($AlCl_3$ assay) in both extracts, together with the use of matched solvent-to-solid ratios across extraction methods.

Comparable extraction-dependent antibacterial effects have been reported in other polyphenol- and saponin-rich plant matrices tested against resistant bacteria, including *Musa balbisiana* fruit extracts enriched in polyphenols and saponins (Hoang et al., 2023) and *Bersama abyssinica* crude extract evaluated against Gram-positive and Gram-negative isolates (Mossie et al., 2024). These findings reinforce the general principle that extraction technique, in addition to plant species, can substantially influence the antibacterial profile of a botanical extract.

This study has several important methodological limitations that should be acknowledged transparently. These limitations are disclosed in the interest of scientific rigor and integrity rather than as grounds for dismissing the principal directional findings. First, the absence of a positive antibiotic control (clindamycin or tetracycline disc) precludes direct comparison of the observed inhibition zones with those produced by clinically used antibiotics. Second, the absence of a vehicle-only negative control means that a small contribution of the CMC vehicle to the observed inhibition zones cannot be definitively excluded. Third, the assays were performed in single replicate (n = 1) rather than in triplicate. Consequently, measures of variability (e.g., standard deviation and 95% confidence intervals) and inferential statistical analyses (e.g. two-way ANOVA with Tukey *post hoc testing*) could not be performed.

Therefore, the differences between maceration and UAE described as "consistent" throughout this manuscript should be interpreted as a recurring directional pattern across concentrations and bacterial species rather than a statistically validated effect. Fourth, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were not determined, limiting quantitative assessment of antibacterial potency. Fifth, phytochemical characterization was qualitative only; total phenolic and flavonoid contents were not quantified, restricting mechanistic interpretation to hypothesis generation rather than confirmed explanation. Sixth, the two extraction protocols employed different solvent-to-solid ratios, introducing a confounding variable that could not be distinguished from the effect of extraction technique itself. Finally, ATCC reference numbers, herbarium voucher information, and ethical-clearance documentation remain to be finalised before resubmission.

Despite these limitations, the consistent dose–response relationship and recurring directional differences between extraction methods provide internal support for the principal finding. Nevertheless, these results should be regarded as preliminary until confirmed by future studies incorporating the controls and analyses described above.

Subject to the methodological refinements outlined above, the present findings support the further development of *P. indica*-based topical formulations as adjunctive anti-acne therapies, particularly in cases where *S. aureus*-associated inflammation contributes to disease pathogenesis. The results also identify maceration as the preferred extraction method for small-scale laboratory preparation. Future studies should incorporate appropriate positive and negative controls, replicate testing, determination of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC), isolation of the principal active fraction

constituents through bioassay-guided fractionation, and in vivo evaluation using animal models of bacterial skin infection.

CONCLUSIONS

Ethanolic extracts of *P. indica* leaves obtained by maceration demonstrated concentration-dependent antibacterial activity and consistently produced larger inhibition zones than extracts obtained by ultrasound-assisted extraction against *C. acnes, S. epidermidis* and *S. aureus* across all six tested concentrations. The largest inhibition zone was observed against *S. aureus* at a concentration of 60% (20.30 mm), which falls within the very strong category according to the Davis–Stout classification. These findings suggest that maceration may be the preferred extraction technique for future development of *P. indica*-based antibacterial preparations. The present preliminary *in vitro* findings identify *beluntas* (*P. indica*) leaf extract as a promising plant-derived candidate for reducing reliance on synthetic antibiotics as a complementary or preventive approach to acne-associated bacterial colonisation. In particular, the results support prioritising the maceration extract for further formulation and evaluation as a topical anti-acne preparation. However, no clinically confirmed therapeutic effect can be inferred from the current data. Confirmatory studies incorporating appropriate positive and negative controls, replicate testing with inferential statistical analysis, minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) determination, phytochemical quantification, and *in vivo* evaluation are required before these findings can be translated into potential clinical application.

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