Indonesian Journal of Pharmacy

VOL 36 (1) 2025: 116–128 | RESEARCH ARTICLE

Antimicrobial Prospects of Domesticated Ornamental Leaf Extracts Against Skin Pathogens

Nurshahira Ezzany Shamshul Hakimi[1], Aliff Zulkarnain Zaini[2] and Mohd Faiz Mustaffa[2,3]*

1. Department of Pharmacy, Hospital Putrajaya, Pusat Pentadbiran Kerajaan Persekutuan, Presint 7, 62250 Putrajaya, Malaysia.
2. Department of Pharmacology and Life Sciences, Faculty of Pharmacy, Universiti Teknologi Mara (UiTM) Selangor, 42300 Bandar Puncak Alam, Selangor, Malaysia.
3. School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, China.

Article Info

Submitted: 23-07-2024
Revised: 12-11-2024
Accepted: 27-11-2024

*Corresponding author
Mohd Faiz Mustaffa

Email:
mohdfaiz@uitm.edu.my /
mohdfaiz@sjtu.edu.cn

ABSTRACT

The increasing popularity of natural plant-based treatments offers an alternative to conventional therapies for various cutaneous infections due to their potential efficacy and lower side effects. Thus, the present study aimed to investigate the antimicrobial and cytotoxic properties of leaf extracts from five selected domesticated ornamental plants against pathogens implicated in acne vulgaris and dermatophytosis. The selected plant leaves were successively macerated with solvents of increasing polarity and the extracts underwent qualitative phytochemical analysis. The antimicrobial activities were evaluated using the broth microdilution method. Extracts with high antimicrobial activity (MIC≤128 µg/mL) were subsequently tested for cytotoxicity on BJ fibroblast cells, and the selectivity index (SI) was calculated. Extraction yields were the highest in *Bougainvilea glabra* (72.04%) and the lowest in *Plumeria obtusa* (21.60%). Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, quinolones, saponins, and coumarins, with phenols found in all extracts. The aqueous extract of *Alamanda cathartica* (AC-Aq) and the methanol extract of *Ixora coccinea* (IC-Met) showed the most potent antibacterial activities against *P. acnes* and *S. epidermidis*, with MIC values of 128 µg/mL and 64µg/mL, respectively. Antifungal activity was most pronounced in AC-Aq against *T. mentagrophytes* and *T. rubrum*, with MIC values of 32 µg/mL. The six active extracts revealed varying degrees of toxicity, with the aqueous extract of *P. obtusa* (PO-Aq) exhibited the least cytotoxicity (CC_{50}:713.31±3.71 µg/mL), while IC-Aq was the most cytotoxic (CC_{50}: 116.72±2.28 µg/mL). AC-Aq demonstrated the highest SI values, indicating effective antimicrobial activity at non-toxic concentrations.

Keywords: Ornamental plants, acne vulgaris, dermatophytosis, antimicrobial activity, cytotoxicity.

INTRODUCTION

Skin-related diseases are the fourth-leading cause of non-fatal disease burden worldwide (Giesey et al., 2021), affecting approximately one-third of the global population across various ages and genders (Prasitpuriprecha et al., 2022; Tizek et al., 2019). Although dermatological diseases, which encompass a wide range of conditions like acne, dermatophytosis, eczema, and psoriasis, are typically not life-threatening, they impose significant emotional and psychosocial burden on patients, diminishing their quality of life (Joseph et al., 2014; Zhang et al., 2019). Affected individuals often struggle with a distorted self-image and lower life satisfaction (Kowalewska et al., 2021).

Among skin-related diseases, acne vulgaris and dermatophytosis represent a significant portion of this burden (Pulsipher et al., 2021; Yakupu et al., 2023). Acne vulgaris, a chronic multifactorial inflammatory condition affecting the pilosebaceous units, is the second-highest contributor to the global burden of skin diseases, accounting for 0.29% of the total disability-adjusted life years (DALYs) worldwide (Pulsipher

et al., 2021). It manifests as inflammatory nodules, pustules, and papules, with the Gram-positive bacteria *Staphylococcus epidermidis* and *Propionibacterium acnes* playing significant roles in pathogenesis. Although these bacteria are generally commensal and non-pathogenic under normal conditions, they become invasive when abnormal skin conditions, such as increased sebum production, or obstruction of pilosebaceous units, arise (Mustarichie et al., 2020).

Dermatophytosis, commonly known as ringworm or tinea, is a group of superficial infections caused by keratinophilic filamentous fungi that invade and proliferate within keratin-rich tissues such as the hair, nails and stratum corneum of the skin. *Trichophyton rubrum* and *Trichophyton mentagrophytes* are the primary causative agents (Rosalie et al., 2021; Tahiliani et al., 2021), leading to symptoms like annular lesions with a raised border and a central healing tendency on the skin (Antuori et al., 2019) and nail abnormalities such as discoloration and thickening (Jartarkar et al., 2022).

Multiple drugs have been introduced to treat acne vulgaris and dermatophytosis (Jartarkar et al., 2022). The management of acne vulgaris includes topical medications (e.g. retinoids, benzoyl peroxide, or clindamycin) and oral medications (e.g. isotretinoin, antibiotics, or hormonal therapy). As for dermatophytosis, the condition is commonly treated with topical or oral antifungals such as azoles and allylamine. Topical administration is generally preferred over systemic administration due to the decreased risk of systemic side effects and toxicity (Răileanu et al., 2023). However, in severe or recalcitrant cases, oral drugs may be considered (Jartarkar et al., 2022). Despite the availability of conventional treatments, overuse and misuse of the drugs have contributed to the development of drug resistance, relapse incidence, and treatment failure (Răileanu et al., 2023).

Considering the diminishing effectiveness of the mentioned treatments against resistant strains, new antimicrobial agents are increasingly necessary (Dembetembe et al., 2023). However, the high costs of synthetic drug development has shifted interest towards plant-based alternatives (Rajput & Kumar, 2020). Plants are well-known for their array of bioactive compounds with potential antimicrobial agents. Among them, ornamental plants, primarily cultivated for aesthetic purposes, have been shown to possess antimicrobial properties against some pathogens (Agbebi et al., 2022; Perveen et al., 2022; Saini et al., 2020). This study focuses on *Allamanda cathartica* (Agbebi et al., 2022; Mannan et al., 2017; Rajamanickam & Sudha, 2013), *Bougainvillea glabra* (Edwin et al., 2007), *Ixora coccinea* (Agbebi et al., 2022), *Pandanus amaryllifolius* (Suwannakul et al., 2018), and *Plumeria obtuse* (Perveen et al., 2022), which have demonstrated antibacterial activity against common bacterial pathogens. Selected for their bioactive potential, these ornamental plants (Figure 1) are commonly found in residential areas across Malaysia. However, despite their known antibacterial properties, their effectiveness against skin pathogens associated with acne vulgaris and dermatophytosis remains underexplored. Thus, this study was done with the aim to evaluate the antimicrobial potential of these leaf extracts against pathogens implicated in acne vulgaris and dermatophytosis, contributing to the growing body of knowledge on the discovery of novel, natural antimicrobial agents for skin infections.

MATERIALS AND METHODS

Plant materials

The five selected domesticated ornamental leaf plants were collected around Kuala Selangor, Selangor, Malaysia. Fresh mature leaves of these ornamental plants were collected from domesticated gardens within the research area and each plant specimen was identified by a botanist. Voucher specimens were deposited in the Herbarium of the Institute of Bioscience, Universiti Putra Malaysia (Figure 1).

Figure 1. Ornamental plants selected for this study with voucher number. (A) *Allamanda cathartica*, (B) *Bougainvillea glabra*, (C) *Ixora coccinea*, (D) *Pandanus amaryllifolius*, and (E) *Plumeria obtuse.*

Preparation of plant extracts

The plant extraction was carried out based on the method described by Jaafar et al., (2023). The collected leaves were thoroughly cleaned, thinly sliced, and dried in a 50°C oven (Venticell, Germany) for 120 hours. The dried leaves, with a final moisture content of approximately 5—10%, were then ground (20,000 RPM, 15 mins) to form a coarse powder in a blender (Waring, USA). For each extract, 250 grams of the initial leaf powder was sequentially soaked in hexane, methanol, and distilled water in a ratio of 1:10 (w/v). The maceration process was performed at room temperature for 72 hours for each solvent. These solutions were then filtered using Whatman No 1 filter paper to obtain the supernatants. The water extract was kept at -80°C for at least 72 hours, followed by lyophilization in a freeze dryer (Labconco, United States), while the hexane and methanol extracts were subjected to rotary evaporation (Buchi, Switzerland). The extracts were thoroughly dried via air drying in a fume hood to remove residual solvents before yield calculations. The percentage yield of crude extract was calculated using Equation 1.

$$\text{Percent yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of dry powder (g)}} \times 100\% \quad \ldots\ldots \textit{Eq. (1)}$$

Phytochemical analysis

The qualitative phytochemical screening is used to detect the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, quinones, saponins and coumarins in the prepared extracts as described by Jaafar et al., (2023).

Inoculum preparation

For dermatophytes, *T. rubrum* (ATCC®-28188) and *T. mentagrophytes* (ATCC®-9533) were sub-cultured on Sabouraud Dextrose Agar (SDA, Life Technologies, United States) at 30°C for 7 to 14 days. The inoculum suspensions were prepared according to Clinical and Laboratory Standards Institute (CLSI) M38-A2 (Clinical Laboratory Standard Institute (CLSI), 2008) guidelines, adjusted to an optical density (OD) of 0.08 to 0.10 at 520 nm, which corresponds to a concentration of 1.0 x 10^8 CFU/mL by using a UV-Vis spectroscopy (Agilent, US) prior analysis.

For acne pathogens, *P. acnes* (ATCC®-6919) and *S. epidermidis* (ATCC®-12228) were sub-cultured on Columbia Sheep Blood Agar (Oxoid, UK) and tryptone soy agar (TSA, HiMedia, India), respectively. The inoculum of *P. acnes* and *S. epidermidis* were prepared according to CLSI M100-S23 (Clinical Laboratory Standard Institute (CLSI), 2021). One colony from the bacterial stock was added to tryptone soy broth (TSB) (HiMidea, India) and incubated in an anaerobic environment (Oxoid AnaeroJar 2.5L Jar) for *P. acnes* or aerobic environment for *S. epidermidis*. Both were kept in an incubator (Nuaire, United Kingdom) for 24 h. The inoculum was adjusted as described previously.

***In-vitro* anti-dermatophyte assay**

Following the CLSI M38-A2 (2008) guidelines, the broth microdilution method was conducted in 96-well round bottom microtiter plates (Singh Gill et al., 2023). A 100 µL quantity of the adjusted inoculum fungal suspension (1.0 x 10^6 CFU/mL) was added to each well containing 100 µL of two-fold serial dilution of leaf extracts, clotrimazole and 5% dimethyl sulfoxide (DMSO), respectively. Growth and sterility controls were included. The inoculated microtiter plates were then incubated at 30°C for 4 to 7 days. Minimum inhibitory concentrations (MICs) were determined visually, with absorbance at 625 nm measured using a microplate reader (Tecan, Switzerland) to determine the IC_{50}. The minimum fungicidal concentrations (MFCs) were identified by plating 10 µL from each treatment well on SDA plates, with a 72-hours incubation at 30°C.

***In-vitro* anti-acne assay**

Antibacterial susceptibility was evaluated via the microdilution broth method as outlined in the CLSI M100-S23 (2021). Serial dilutions of leaf extracts and clindamycin (positive control) were prepared in TSB across 96-well plates. Each well was inoculated with 100 µL of bacterial suspension (1.0 x 10^6 CFU/mL) for *P. acnes* and *S. epidermidis*, and plates were incubated at 37°C for 72 hours under anaerobic conditions for *P. acnes* and standard conditions for *S. epidermidis*. MICs were visually assessed, and IC50 values were analyzed at 625 nm. Minimum bactericidal concentrations (MBCs) were determined by culturing 10 µL from each well on specified agar, with 24-hour incubation to confirm growth inhibition.

Following the classification by Silva et al., (2013), plant extracts were categorized based on their MIC values as follows: highly potent (MIC < 100 µg/mL), potent (MIC 100–500 µg/mL),

moderately potent (MIC 501–1000 µg/mL), and less potent (MIC > 1000 µg/mL).

Cytotoxicity assay

The highly active extracts (MIC ≤ 128 µg/mL) against dermatophytes and acne strains were further assessed for cytotoxicity using the MTT assay. Human foreskin BJ fibroblast cells (ATCC®CRL-2522) were maintained in Eagle's Minimum Essential Medium (MEM, Life Technologies) supplemented with 10% fetal bovine serum and penicillin (Life Technologies) at 37°C in a humidified atmosphere (95% O_2/5% CO_2). Each potent extract, in concentrations ranging from 1.95 to 4000 µg/mL was added to 96-well plates containing 100 µL of fibroblast cells (2.5 x 10^4 cells/mL). Sterility and negative control were included. Plates were incubated for 72 h in a humidified 5% CO_2 atmosphere. Following incubation, 20 µL of MTT solution (5 mg/mL) was added and further incubated for another 3 h. After centrifuging at 1500 RPM for 5 mins, the supernatant was removed, and DMSO was added to dissolve formazan crystals. Absorbance was measured at 570 nm using a spectrophotometer. Cytotoxicity was determined by plotting dose-response curves and calculating the concentration that inhibited 50% of cell growth (CC_{50}). Assays were conducted in triplicate.

Selectivity index (SI)

To evaluate the therapeutic potential of the extracts, the selectivity index (SI) was calculated using the following Equation 2.

$$\text{Selectivity Index (SI)} = \frac{CC_{50} \text{ of extracts against human foreskin BCL – 2522 Cells}}{IC_{50} \text{ of extracts against microbial strains}}$$

……Eq. (2)

According to Indrayanto et al., (2021), an extract with an SI value of ≥ 10 is generally considered to have a high therapeutic index, indicating a wide safety margin for therapeutic use.

Statistical analysis

The experimental values of MIC, MBC and MFC were expressed as the means of three consistent replicates of the plates. The MIC_{index} of each extract was calculated against each test strain as shown in Equation 3.

$$\text{MIC index} = \frac{\text{MFC/MBC}}{\text{MIC}} \quad \text{……. } Eq.\ (3)$$

Antimicrobials were classified as bactericidal or fungicidal if the MBC/MIC or MFC/MIC ratio was ≤ 4, and as bacteriostatic or fungistatic if the ratio was > 4 (Pfaller et al., 2004). Data were analyzed using SPSS, and statistical differences were assessed by two-way analysis of variance (ANOVA) with Tukey's multiple comparison test. The results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Percentage of extraction yield

The extraction yields of the five selected plants, obtained via maceration using hexane, methanol and distilled water (Table I). *B. glabra* (BG) had the highest overall yield, ranging from 18.06% to 31.89%, while *P. obtusa* (PO) showed the lowest, with yields from 3.69% to 9.19%. Methanol extraction yielded the highest percentages for *A. cathartica* (AC-Met: 25.79%), *I. coccinea* (IC-Met: 22.08%), and *P. amaryllifolius* (PA-Met: 17.08%), whereas hexane extraction was most efficient for *B. glabra* (BG-Hex: 31.89%). The macerations method, using solvents with increasing polarity (hexane, methanol, and distilled water), offers efficient extraction with basic equipment, and preserves thermosensitive compounds (González-Fernández et al., 2020). This approach enhances the isolation of diverse bioactive compounds by maximizing their partitioning across solvents of different polarities (Fotsing et al., 2021). The variation in extraction yield, such as the high yield observed in *B. glabra* (>70%) compared to previous reports indicating lower yields (<10%) (Riaz et al., 2021), may be attributed to differences in extraction procedures and the geographical origin of the plants (Velázquez-Martínez et al., 2022). Additionally, solvent polarity plays a crucial role, with methanol favoring the extraction of polar compounds, as seen in and solvent capacity to dissolve specific plant *A. cathartica*, *I. coccinea* and *P. amaryllifolius*, which aligns with prior studies on *I. coccinea* (Upadhyay et al., 2014), and *A. cathartica* (Fartyal, 2016). Similarly, the aqueous extraction yield was highest for *P. obtuse* (PO-Aq), consistent with Kamran et al., (2020). According to Truong et al., (2019), higher yields in methanolic and aqueous extracts are likely due to the abundance of polar compounds in these plants. The substantial yield observed in the BG-Hex may reflect its higher concentrations of non-polar compounds.

Table I. Percentage of extraction yield and phytochemical screening.

Plant	Extract	Extraction yield (%)	Phytochemical Constituents							
			AL	FL	TN	TA	PH	QN	SP	CM
Allamanda cathartica (AC)	Hexane (AC-Hex)	8.25	-	+	+	-	+	+	+	+
	Methanol (AC-Met)	25.79	-	+	+	-	+	-	-	+
	Aqueous (AC-Aq)	16.16	-	+	+	-	+	+	+	+
Bougainvillea glabra (BG)	Hexane (BG-Hex)	31.89	+	+	+	+	+	+	-	+
	Methanol (BG-Met)	18.06	+	+	-	+	+	-	-	+
	Aqueous (BG-Aq)	22.09	+	+	+	+	+	+	-	+
Ixora coccinea (IC)	Hexane (IC-Hex)	7.86	+	+	+	-	+	-	-	-
	Methanol (IC-Met)	22.08	+	+	+	+	+	+	+	-
	Aqueous (IC-Aq)	19.84	+	+	+	+	+	-	+	-
Pandanus amaryllifolius (PA)	Hexane (PA-Hex)	5.86	-	+	+	-	+	-	-	-
	Methanol (PA-Met)	17.08	+	+	+	-	+	-	+	+
	Aqueous (PA-Aq)	3.84	-	+	+	-	+	-	-	+
Plumeria obtusa (PO)	Hexane (PO-Hex)	3.69	+	-	+	-	+	-	-	-
	Methanol (PO-Met)	8.72	+	-	+	-	+	+	-	-
	Aqueous (PO-Aq)	9.19	+	-	+	-	+	-	-	-

Note: AL: alkaloids; FL: flavonoids; TN: terpenoids; TA: tannins; PH: phenols; QN: quinones; SP: saponins; CM: coumarins. + present; - absent.

Phytochemical Screening of Leaf Extracts

The phytochemical screening of the selected leaf extracts revealed diverse phytoconstituents, including alkaloids, flavonoids, terpenoids, tannins, phenols, quinolones, saponins and coumarins (Table I). *B. glabra* exhibited the highest total extraction yield (72.04%) and, along with *I. coccinea* (49.78%) displayed the widest range of phytoconstituents, with only saponins in *B. glabra* and coumarins in *I. coccinea* absent from their respective extracts. Phenols were detected in all tested extracts, while terpenoids were absent only in the methanol extract of *B. glabra.* In contrast, *A. cathartica* (50.2%) contained nearly all tested phytoconstituents, except alkaloids and tannins. *Plumeria obtusa*, with the lowest total extraction yield (21.6%), presented the most limited profile, lacking flavonoids, tannins, saponins, and coumarins across all extracts. The extraction yield appears to correlate with the quantities and types of bioactive constituents in each plant material, reflecting species-specific differences in phytochemical composition (Aliabasi et al., 2023; Fan et al., 2023; Wong-Deyrup et al., 2021).

Literature reports indicate that *B. glabra* contains high levels of betacyanin (Saleem et al., 2021), along with flavonoids such as quercetin, flavanones, and flavanols, as well as nerolidol (Ogunwande et al., 2019). These compounds are recognized for their significant antibacterial properties (Ogunwande et al., 2019; Saleem et al., 2021). In contrast, *P. amaryllifolius* contains a diverse array of bioactive compounds, including gallic acid, catechin, caffeic acid, myricetin, luteolin, piperidine, and quercetin, many of which are documented for their antidermatophytic effects (Wang et al., 2024). Based on these phytochemical profiles, extracts from both plants were subjected to antimicrobial assays targeting acne-causing and dermatophytic pathogens, enabling the correlation of specific phytochemicals with observed anti-acne and antidermatophytic activities.

Antimicrobial Activities of Leaf Extracts

The anti-acne activity results (Table II) indicated that, of the 15 extracts tested, five demonstrated potent activity (MIC: 128–256 µg/mL) against *P. acnes*. Among these, the AC-Aq and IC-Met exhibited the strongest inhibition (MIC: 128 µg/mL), followed by AC-Met, IC-Aq and PO-Aq, each with an MIC of 256 µg/mL. None of these extracts achieved the threshold for high potency (MIC< 100 µg/mL). For *S. epidermidis*, six extracts were classified as potent, with two (AC-Aq and IC-Met) exhibiting highly potent activity (MIC: 64 µg/mL), comparable to the positive control, clindamycin (MIC: 16 µg/mL, $p > 0.05$). This strong inhibition of *S. epidermidis* by AC-Aq and IC-Met highlights the potential of these extracts as natural alternatives to conventional anti-acne agents.

Table II. *In-vitro* anti-acne and anti-dermatophyte activities of leaf extracts

Extract/ Drug	Acne pathogens						Dermatophyte pathogens					
	P. acnes ATCC®-6919			*S. epidermidis* ATCC®-12228			*T. mentagrophytes* ATCC®-9533			*T. rubrum* ATCC®-28188		
	MIC	**MBC**	**M_i**	**MIC**	**MBC**	**M_i**	**MIC**	**MFC**	**Mi**	**MIC**	**MFC**	**Mi**
AC-Hex	1024^{d}	NI	-	512^{c}	NI	-	512^{b}	NI	-	512^{b}	NI	-
AC-Met	256^{b}	512^{c}	2	**128^{b}**	256^{b}	2	**32^{a}**	64^{a}	2	**64^{a}**	128^{a}	2
AC-Aq	**128^{b}**	256^{b}	2	**64^{a}**	64^{a}	1	**32^{a}**	32^{a}	1	**32^{a}**	64^{a}	2
BG-Hex	512^{c}	1024^{d}	2	512^{c}	1024^{c}	2	1024^{c}	2048^{d}	2	512^{b}	2048^{d}	4
BG-Met	1024^{d}	NI	-	2048^{e}	NI	-	NI	NI	-	NI	NI	-
BG-Aq	NI	NI	-	NI	NI	-	NI	NI	-	NI	NI	-
IC-Hex	1024^{d}	NI	-	1024^{d}	2048^{d}	2	1024^{c}	NI	-	1024^{c}	NI	-
IC-Met	**128^{b}**	256^{b}	2	64^{a}	128^{a}	2	512^{b}	512^{b}	1	256^{b}	512^{b}	2
IC-Aq	256^{b}	256^{b}	1	**128^{b}**	256^{b}	2	1024^{c}	1024^{c}	1	512^{b}	1024^{c}	2
PA-Hex	512^{c}	2048^{e}	4	256^{b}	1024^{c}	4	256^{b}	512^{b}	2	**128^{b}**	128^{a}	1
PA-Met	1024^{d}	NI	-	512^{c}	NI	-	512^{b}	1024^{c}	2	256^{b}	512^{b}	2
PA-Aq	2048^{e}	NI	-	512^{c}	2048^{d}	4	NI	NI	-	2048^{d}	NI	-
PO-Hex	NI	NI	-	NI	NI	-	NI	NI	-	NI	NI	-
PO-Met	2048^{e}	NI	-	2048^{d}	NI	-	1024	NI	-	1024^{c}	NI	-
PO-Aq	256^{b}	512^{c}	2	**128^{b}**	256^{b}	2	256^{b}	256^{b}	1	512^{b}	512^{b}	1
Clindamycin	2^{a}	4^{a}	2	16^{a}	64^{a}	4	-	-	-	-	-	-
Clotrimazole	-	-	-	-	-	-	16^{a}	64^{a}	4	8^{a}	64^{a}	8

Note: MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration; MFC – minimum fungicidal concentration; Mi – MIC index; NI – no inhibitory activity at concentrations ≤ 2048 µg/mL; "-" – not determined. "a-e" Values within a column with different superscripts are significantly different from each other at $p < 0.05$. Numbers in bold indicate highly potent and selected for further analysis.

Conversely, the extracts BG-Met, IC-Hex, PO-Hex, and PO-Met showed no inhibitory activity against either *P. acnes* or *S. epidermidis* at concentrations up to 1000 µg/mL, suggesting limited antibacterial constituents in these solvent-specific extractions.

The anti-acne activity observed for the AC-Aq and IC-Met in this study represents a novel finding, as no prior studies have evaluated these specific extracts against acne-causing pathogens. This lack of precedence highlights the unique contribution of our work to the field of natural anti-acne agents. Based on anti-acne efficacy, five extracts (AC-Aq, AC-Met, IC-Aq, IC-Met, and PO-Aq) were selected for further investigation.

In terms of antifungal activity, AC-Aq demonstrated the strongest inhibitory effects, with MIC values of 32 µg/mL against both *T. mentagrophytes* and *T. rubrum* (Table II). The AC-Met showed similar efficacy against *T. mentagrophytes* (MIC: 32 µg/mL) but required a higher concentration (MIC: 64 µg/mL) to inhibit *T. rubrum*. As expected, the positive control, clotrimazole, displayed potent activity against both fungi, with MIC values of 16 µg/mL and 8 µg/mL, respectively. The antifungal effects of AC-Aq and AC-Met were comparable to clotrimazole ($p > 0.05$), indicating their potential as effective antifungal agents. Additionally, PA-Hex exhibited moderate antifungal activity against *T. mentagrophytes* (256 µg/mL) and *T. rubrum* (128 µg/mL). In contrast, BG-Aq, BG-Hex and PO-Hex showed no detectable antifungal activity against the dermatophyte strains, suggesting a limited presence of antifungal constituents in these extracts. Based on these results, three extracts (AC-Aq, AC-Met, and PA-Hex) were selected for further cytotoxicity evaluation due to their promising antifungal potency.

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) assessments provided further insights into the bactericidal and fungicidal nature of the extracts. All six selected extracts (AC-Met, AC-Aq, IC-Met, IC-Aq, PA-Hex, and PO-Aq) were classified as bactericidal or fungicidal against their respective strains, with MBC/MIC or MFC/MIC ratios ≤ 4. This indicates that these extracts not only inhibit growth but also possess the capability to kill the microbial cells, enhancing their potential as effective antimicrobial agents.

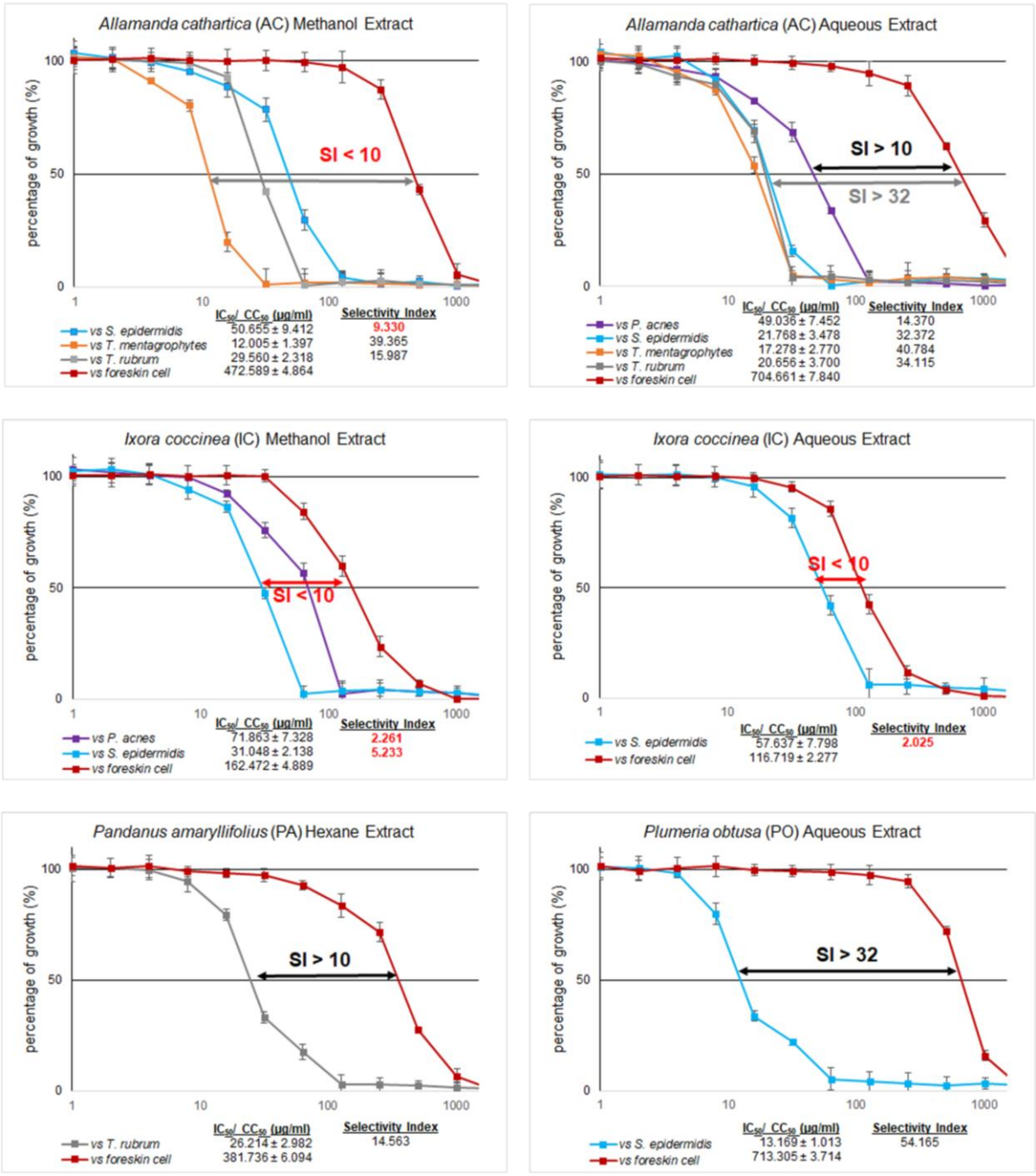

Figure 2. IC_{50}, CC_{50} and selectivity index of active leaf extracts against tested microbial strains and human skin cell line.

These methanol and water extracts, rich in polar compounds such as flavonoids, phenolics, and terpenoids, exhibit microbicidal activities. Among these compounds, quercetin—a widely occurring flavanol—has been shown to inhibit various drug-resistant bacteria and fungi (Mandal & Domb, 2024). This effectiveness is attributed to its ability to disrupt cell walls, cause membrane damage, and intercalate with DNA, leading to the inhibition of nucleic acid and protein synthesis, and preventing biofilm formation (Macêdo et al., 2024; Salehi et al., 2020; Veiko et al., 2023). The potent antimicrobial

activity observed in these polar extracts supports the role of quercetin and similar compounds in effectively inhibiting pathogen growth, aligning with the higher efficacy of polar extracts compared to non-polar extracts.

Among the tested extracts, AC-Aq and AC-Met exhibit particularly high potency against the tested pathogens, likely due to their rich bioactive profile, which includes steroids, terpenoids, flavonoids, coumarins, lignans, and particularly iridoids (de F Navarro Schmidt et al., 2006). Iridoids, a class of compounds documented in *A. cathartica* extracts for their anti-inflammatory, antibacterial, antifungal, and antioxidant properties, which align with the potent anti-acne and anti-dermatophyte activities observed in these two extracts (Petricevich & Abarca-Vargas, 2019). Compounds such as isoplumericin, allamandin, and plumieride have been identified in *A. cathartica* (Bonomini et al., 2017), with plumieride recognized for its potent antidermatophytic activity against *Epidermophyton floccosum* and *Microsporum gypseum* (Tiwari et al., 2002). Additionally, plumieride has shown potential in treating skin candidiasis by exerting anti-inflammatory effects, increasing iNOS expression, and reducing the levels of proinflammatory genes such as TNF-α, IL-1β, and NF-κB (El-Shiekh et al., 2024). The presence of flavonoids, phenolics, and terpenoids in AC-Aq and AC-Met further supports their efficacy against acne and dermatophytic pathogens. These findings suggest that the bioactive profile of *A. cathartica*, especially its iridoid content, significantly enhances its therapeutic potential for treating skin infections.

Cytotoxic effect and selective index of active leaf extracts

To ensure the safety of these extracts following their promising antimicrobial efficacy, we conducted cytotoxic assessment on human foreskin BJ fibroblasts, representing normal skin cells, expressed as the median inhibitory concentration (CC_{50}). The findings revealed that all tested maintained acceptable levels of toxicity, with CC_{50} values ranging from 116.72±2.28 µg/mL to 713.31±3.71 µg/mL (Figure 2). According to the (de Lima et al., 2019), extracts can be classified as nontoxic (CC_{50} > 1000 µg/mL), poorly toxic (CC_{50}: 500–1000 µg/mL), moderately toxic (CC_{50}: 100–500 µg/mL) or very toxic (CC_{50} < 100 µg/mL). None of the tested extracts were classified as highly toxic (CC_{50} < 100 µg/mL), which has their potential for safe application at therapeutic concentrations. Specifically, AC-Aq (CC_{50} = 704.66±7.84 µg/mL) and PO-Aq (CC_{50} = 713.31±3.71 µg/mL) were classified as poorly toxic, while the remaining four tested extracts were classified as moderately toxic, with cytotoxicity levels increasing in the order of AC-Met (472.59±4.87 µg/mL) < PA-Hex (381.74±6.09 µg/mL) < IC-Met (162.47±4.89 µg/mL) < IC-Aq (116.72±2.28 µg/mL).

To better assess the therapeutic applicability of these extracts, selectivity index (SI) values were calculated by comparing the CC_{50} (cytotoxic concentration) with the IC_{50} (antimicrobial concentration) for each pathogen. An SI value of 10 or greater is typically considered indicative of favorable selectivity toward microbial pathogens over host cells. Among the six extracts tested, the AC-Aq demonstrated the highest SI values across multiple pathogens, including *P. acnes* (SI: 14.4), *T. mentagrophytes* (SI: 40.8) and *T. rubrum* (SI: 34.1). Meanwhile, the best selectivity for *S. epidermidis* was observed in the PO-Aq, with a SI value of 54.2. In contrast, low SI values were exhibited by the IC-Met and IC-Aq against their respective pathogens (SI < 10), indicating limited selectivity and a higher likelihood of general cytotoxicity rather than specific antimicrobial effects. These low SI values suggest that IC-Met and IC-Aq may not be suitable for therapeutic application due to their low safety margin.

A. cathartica is known to contain toxic iridoid lactones that have been reported to induce dermatitis symptoms (Maroyi, 2012). However, the results from the current cytotoxicity study indicate low cytotoxic activity of both aqueous and methanol extracts on BJ fibroblast cells. *In vivo* studies on AC-Aq leaf extract demonstrated its safety and effectiveness in promoting wound healing, with results showing faster wound contraction, increased skin strength, elevated collagen synthesis, and reduced inflammation (Nayak et al., 2006). These findings highlight AC-Aq's potential for safe skin repair, underscoring the importance of proper formulation and concentration to balance the extract's efficacy with skin tolerability, making it suitable for dermatological applications (Mehta et al., 2017).

CONCLUSIONS

The findings demonstrated that methanol and aqueous extracts of *B. glabra*, *A. cathartica*, and *I. coccinea* contain higher levels of bioactive compounds compared to hexane extracts. Phytochemical analysis identified various compounds, including flavonoids, terpenoids, phenols, coumarins, quinolones, and saponins,

which are likely to contribute to the observed antimicrobial properties. The AC-Aq exhibited highly potent activity against all tested strains, except for *P. acnes*, against which it showed potent antibacterial activity. Importantly, the high selectivity index of AC-Aq further supports its therapeutic potential for treating skin infections. However, further studies are needed to isolate and identify the specific compounds responsible for these antimicrobial activities.

ACKNOWLEDGMENTS

The authors would like to acknowledge the financial support provided by Universiti Teknologi MARA, Malaysia (Grant No. 600-UITMSEL (PI. 5/4) (150/2022)) and the Faculty of Pharmacy for providing state-of-the-art facilities to complete the research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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