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Bioactivities of Leaf Extracts from *Urceola polymorpha* (Pierre) D.J.Middleton & Livsh., *Hyptis suaveolens* (L.) Poit, and *Passiflora foetida* L. Leaf: Antioxidant, Antibacterial, Cytotoxic and Anti-tyrosinase Potential with Molecular Docking Analysis

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Abstract

Weeds are naturally occurring plants, often lacking economic value and potentially harming cash crops. However, some weeds contain beneficial compounds, and with proper research, they could be utilized to their fullest potential. In this study, leaf weeds extracted from *Urceola polymorpha* (Pierre) D.J.Middleton & Livsh., *Hyptis suaveolens* (L.) Poit, and *Passiflora foetida* L. were analyzed for their phenolic content, antioxidant activity, antibacterial activity, and cytotoxicity. Among these, *P. foetida* exhibited the highest phenolic content at 12.99 mg/g dry extract. The extract of *H. suaveolens* demonstrated the strongest DPPH radical scavenging activity, with an IC_{50} value of 0.07 mg/mL, while *P. foetida* had the highest activity in the FIC assay with an IC_{50} is 0.35 mg/mL. In antibacterial assays, *P. foetida* had the lowest minimum inhibitory concentrations (MIC) of 0.50 mg/mL against *B. subtilis*, with scanning electron microscopy revealing cell membrane damage. All extracts were found to be non-toxic to fibroblast (3T3), epithelial (Vero), and macrophage cells (RAW 264.7). LC-MS analysis identified flavonoids as the main phenolic compounds in *P. foetida*. Furthermore, *P. foetida* showed tyrosinase inhibition with an IC_{50} of 64.04 mg/mL, supported by molecular docking studies ($\Delta G_{binding}$ between –8.12 to –7.38 kcal/mol). This research highlighted these weed extracts as potential sources of bioactive compounds with valuable biological properties.

Keywords: Antibacterial, Antioxidant, Total phenolic, Weed, Anti-tyrosinase, Molecular docking

Introduction

Many contemporary health issues stem from unclean environments, where free radicals, toxins, viruses, fungi, and bacteria thrive. These factors can trigger various diseases, including cancer and lung disease from free radicals, as well as infections like inflammation and food poisoning from microorganisms. These challenges significantly impact healthcare and overall wellbeing. Today, the diversity of bacteria that cause diseases in humans has evolved, leading to increased resistance to medications. Consequently, treatments against these pathogens are becoming less effective, resulting in higher healthcare costs, lost time, and tragically, loss of life. Thus, identifying compounds that can inhibit the growth of microorganisms or neutralize free radicals is imperative. While the challenges posed by free radicals and microorganisms continue to impact health, a growing body of research suggests that natural compounds, particularly those found in plants, could offer effective solutions.

Although the human body has an innate defense mechanism to counteract pollutants. However, an excess of these compounds can impair the detoxification process, rendering it less efficient. This inadequate necessitates external interventions, such as antioxidants or antimicrobial agents. Notably, polyphenols and flavonoids are prominent antioxidant groups found in fruits and plants offer potential health benefits [1]. Similarly, compounds like ursolic acid and oleanolic acid known for their antibacterial properties, are commonly present in fruits and vegetables [2]. There is growing interest locally cultivated vegetables that may shield against disease-causing free radicals, as they are rich in antioxidants [3]. For instance, leaf extracts from *Tiliacora triandra* have shown efficacy in inhibiting *Escherichia coli*, with phenolic component identified [4]. Additionally, leaf extracts from *Musa* sp., particularly those containing ethyl acetate, exhibit the ability to suppress both free radicals and microorganisms such as *E. coli*, *Pseudomonas aeruginosa*, and *Citrobacter* sp. [5]. Thailand's rich floral diversity has spurred investigations into various active compounds sourced from endemic plants, aiming to uncover novel biological agents and natural reservoirs of antioxidants, antibacterial, and anticancer properties [3,6]. However, while many studies have focused on fruits, vegetables, and medicinal plants, there remains a significant research gap concerning weeds, especially edible weeds that are traditionally consumed but scientifically underexplored. Only a limited number of studies have reported the antimicrobial activity of a few weed species, such as *Acalypha indica* L., *Ageratum conyzoides*, *Phyllanthus niruri* L., and *Amaranthus spinosus* [7], and these reports often lack detailed chemical characterization or comprehensive biological evaluations. Furthermore, there is a lack of comparative studies assessing the antioxidant, antibacterial, and enzyme-inhibiting potential of multiple edible weeds using standardized in vitro and in silico approaches. As a result, the functional value of weeds remains underutilized and poorly understood in the scientific community. A weed is an unwanted plant that interferes with human activities, posing hazards, aesthetic issues, or management challenges in farms, gardens, and urban spaces [8]. Resorting to chemical means for weed eradication can exacerbate environmental degradation. Therefore, exploring alternative strategies to mitigate the impact of weeds is warranted.

As consumers increasingly prioritize environmental sustainability and personal health, interest in natural extracts, including those from weeds, is growing. Weeds are naturally occurring plants that can thrive without human intervention, potentially possessing advantageous qualities such as antioxidant activity and resistance to diseases and insects. The 3 weeds studied in this study were *Urceola polymorpha* (Pierre) D.J.Middleton & Livsh., *Hyptis suaveolens* (L.) Poit and *Passiflora foetida* L. since they are edible and traditionally used. This study aims to assess the quantity of phenolic compounds in leaf extracts of *U. polymorpha* (Pierre) D.J.Middleton & Livsh., *H. suaveolens* (L.) Poit, and *P. foetida* L. focusing on their antioxidants, antibacterial properties, and cytotoxicity. Furthermore, the study investigates the phytochemical and tyrosinase inhibitory activity in the *P. foetida* leaf extract. The binding efficiency of active compounds against tyrosinase was examined based on silico molecular docking simulation. This approach is a computational method used to predict ligand-protein binding interactions and evaluate binding affinity, providing insights into binding conformation, binding mode, and residues involved in protein-ligand interactions [9]. The conceptual framework of research

was shown on **Figure 1**. This research provides insights into utilizing weeds for their potential benefits in inhibiting pathogens and combating free radicals. If certain weeds exhibit favorable properties, they could be further developed into valuable economic crops in the future.

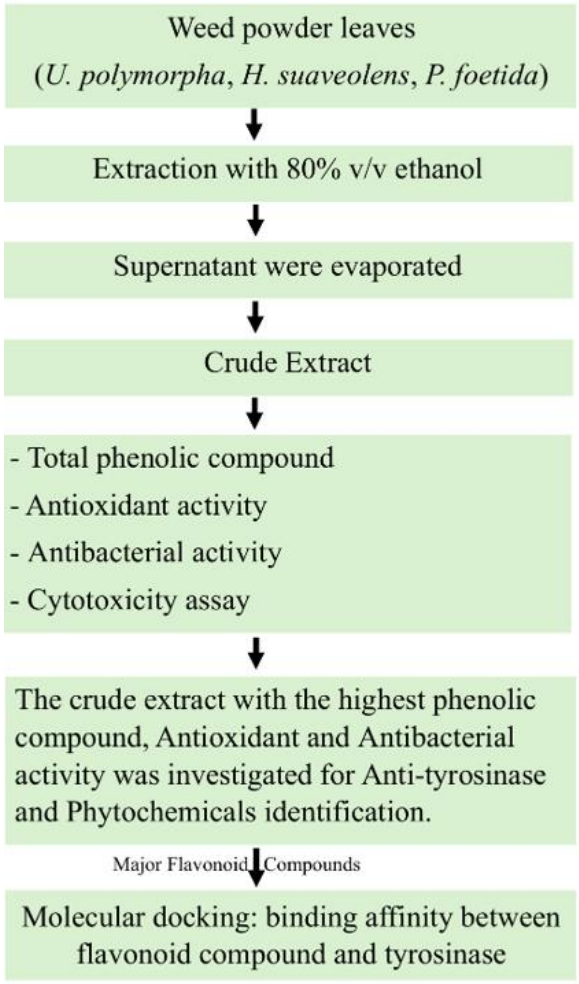

Figure 1 Conceptual framework for research.

Figure 2 Leaf characteristics of (A) *U. polymorpha*, (B) *H. suaveolens*, and (C) *P. foetida*.

Materials and methods

Materials

Three weed species were used: *U. Polymorpha* (Som-Lom), *H. Suaveolens* (Mang-Luk-Ca), and *P. foetida* (Tum-Lung-Pa) (**Figure 2**). They were collected from different locations in Sakon Nakhon province, Thailand. The collection sites were as follows: Site 1 (*U. polymorpha*) at latitude 17.296609, °N, longitude 104.113686°E; Site 2 (*H. suaveolens*) at latitude 17.294313°N, longitude 104.115973°E, and Site 3 (*P.*

foetida) at latitude 17.279691°N, longitude 104.114415°E. All samples were harvested during the rainy season. Five different bacteria were included: 1 Gram-negative (*E. coli* TISTR 527); and 4 Gram-positive strains (*Staphylococcus aureus* TISTR2329, *B. subtilis* TISTR1248, *B. cereus*, and *Staphylococcus epidermidis*). This selection provided a representative sample of pathogenic bacteria relevant to gastrointestinal diseases, skin infections, and complications from weakened immune systems. Fibroblast cell line (3T3), African green monkey epithelial cell line (Vero), and the murine macrophage cell line (RAW 264.7) were sourced from the American Type Culture Collection (ATCC; USA), chosen for their established roles in biological and pharmacological research. Analytical chemicals were utilized for studies involving phenolic compounds, antioxidant activity, antibacterial properties, cytotoxicity, anti-tyrosinase activity, and phytochemical analysis.

Sample preparation and extraction

Each weed sample was thoroughly washed using tap water, dried at 50 °C for 2 h, and then were grounded into a fine powder using a household herbal grinder. A total of 100 g of each type of weed powder was mixed in 300 mL of 80 % v/v ethanol and shaken for 24 h at 150 rpm. The supernatant was separated using centrifugation (3500 rpm for 20 min), and the solvent was removed with a rotary evaporator [10]. All crude extract were stored at 4 °C for later analysis of phenolic components, antioxidant properties, antibacterial effects, cytotoxicity, anti-tyrosinase activity, and phytochemicals.

Total phenolic compound content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method [11,12]. Crude extract (50 µL) was mixed with 80 µL of 10 % Folin-Ciocalteu reagent and 150 µL of 7 % sodium carbonate in 96-well plate, incubated in the dark at room temperature for 2 h. Gallic acid serve as the standard and the absorbance was measured at 765 nm, using methanol served as the control. The standard curve was used for quantification, expressed as milligrams of gallic acid equivalent (mgGAE) per gram of dry extract. Determination of TPC using Eq. (1).

$$TPC = cv/m \quad (1)$$

Where TPC is total phenolic content (mg/g dry extract), c is the concentration of gallic acid established from the calibration curve (mg/mL), v is the volume of the extract (mL), and m is the dry weight of the weed extract (g).

Antioxidant activity

Antioxidant activity was evaluated based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the ferrous ion chelating (FIC) method.

The DPPH method was performed as described in previous reports [10-12]. The crude extract concentration used ranged from 0.01 to 1.00 mg/mL. In brief, 50 µL of crude extract was combined with 0.1 mM DPPH methanolic solution in a 96-well plate. The mixture was thoroughly mixed and incubated in the dark at room temperature for 20 min. The absorbance was measured at 515 nm. A DPPH radical solution in the extraction solvent served as the negative control while gallic acid served as positive control. The scavenging activity was calculated using Eq. (2).

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (2)$$

Where Acontrol is the absorbance of the DPPH radical solution without crude extract and Asample is the absorbance of the DPPH radical solution with crude extract. The half maximum inhibitory concentration (IC_{50}) was used to express the results.

The FIC approach was applied following the guidelines established by Preecharram *et al*. [13]. Briefly, this entailed creating a reaction mixture of 25 µL of 2 mM $FeCl_2$, 800 µL of 70 % ethanol and 250 µL of crude extract at different concentrations. The crude extract concentration used ranged from 0.10 to 15.00 mg/mL. The liquids were thoroughly mixed and then incubated for 5 min. Next, 100 µL of 5 mM ferrozine was added and the mixture was again mixed before an additional 5 min of dark incubation at room temperature. Then, using 70 % ethanol as a blank, Ethylenediaminetetraacetic acid (EDTA) as a positive control, the absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm. Chelating ability was determined using Eq. (3).

$$\text{Chelating ability (\%)} = [(\text{AcFIC} - \text{AsFIC})/\text{AcFIC}]\times 100 \quad (3)$$

Where AcFIC is the FIC reaction mixture's absorbance without the crude extract and AsFIC is the FIC reaction mixture's absorbance with crude extract. The results were expressed as IC_{50}.

Antibacterial activity

The quantitative bacterial inhibition test uses a broth dilution assay to calculate the percentage reduction in bacteria [14]. In the current study, the bacteria were cultured in nutrient broth at 37 °C for 6 - 8 h. Following this, the absorbance of the bacterial solution was measured at a wavelength of 600 nm. Subsequently, it was diluted to match the bacterial density of McFarland No.0.5 (10^8 CFU/mL). Then, to achieve a final culture quantity of 10^6 CFU/mL, each concentration of extract was combined with the diluted bacterial solution to produce final extract concentrations of 1.00, 0.50, and 0.25 mg/mL. After a 24 h incubation period at 37 °C, the absorbance of each of the combined solutions was measured at a wavelength of 600 nm. The positive control and negative control underwent the same procedure as the sample; however, the extract was replaced with the antibiotic clindamycin and 10 % ethanol, respectively.

The percentage of bacterial decrease was determined based on Eq. (4).

$$\text{Bacterial decrease (\%)} = [(B - A)/B]\times 100 \quad (4)$$

Where A is the absorbance value of the sample, and B is the absorbance value of the negative control.

The minimum inhibitory concentrations (MIC90) value is the lowest concentration of extract that can reduce bacteria by 90 % compared to the control.

Effects of extracts on morphological microbial cells

Scanning electron microscopy (SEM) was utilized to analyze the morphological characteristics of bacterial cells following the methodology outlined by Kommanee *et al*. [15]. *B. subtilis* was cultured in nutrient broth, from which cells were harvested during the logarithmic growth phase, followed by centrifugation at 2,500× g for 10 min. Subsequently, the cells were given 2 washes with phosphate buffer (pH 7.4). After dissolving the cell precipitate, the final concentration reached 10^6 CFU/mL. Next, each weed extract was incubated with 500 µL of *B. subtilis* cell suspension at 37 °C for 2 h. The incubated bacteria were fixed using 2.5 % glutaraldehyde in phosphate buffer at pH 7.4. Cellulose filter paper was immersed carefully in the cell solution. Then, the fixed materials were dried using sequential immersion for 15 min each in ethanol solutions of increasing concentration (30, 50, 70, 90, and finally 95 %). Subsequently, an ion sputtering coater (Hitachi; MC1000; Japan) was used to apply a gold coating to the dried specimens, which were then analyzed using SEM (Hitachi; TM4000 Plus; Japan). Similar procedures were followed for the negative controls, except that Phosphate buffer saline (PBS) at pH 7.4 was used to culture the bacterial cells instead of weed extracts.

Cytotoxicity assay

The cytotoxic activities of *U. polymorpha*, *H. suaveolens*, and *P. foetida* leaf extracts were appraised on RAW 264.7, Vero, and 3T3 cells using an MTT assay, with slight modifications from the methodology described by Preecharram *et al.* [10] and Situmeang *et al.* [16]. Briefly, each type of cell was cultured in a 96-well plate at a density of 10^4 cells per well and incubated at 37 °C in a 5 % CO_2 atmosphere for 24 h. Following the incubation, the cells were treated with various concentrations of each crude extract (62.5, 125, 250, 500 and 1000 ug/mL) for another 24 h. After each treatment, the medium was replaced and 0.5 mg/mL of MTT was added to the wells. Then, the cells were incubated at 37 °C in a 5 % CO_2 atmosphere for 1 h. Subsequently, the medium was removed, and the resulting formazan precipitate was dissolved in dimethyl sulfoxide. The absorbance of the dissolved formazan was measured at 570 nm using a microplate reader (PerkinElmer; En-Sight; USA). Cell viability was calculated by comparing the absorbance of the treated cells with that of control cells using Eq. (5).

$$\text{Cell viability (\%)} = [(Ax - Ay)]/(Az - Ay)]\times 100 \quad (5)$$

Where Ax is the average absorbance of the cells treated with the extract, Ay is the average absorbance of the blank medium, and Az is the average absorbance of the cell control.

Phytochemicals identified using LC-MS

Compounds in the extracts were separated and detected using an Agilent 1290 Infinity LC system (Agilent Technologies; Santa Clara, CA, USA) connected to an Agilent 6540 series QTOF-MS which was outfitted with a diode array detector and an electrospray ionization (ESI) source.

The phytochemical components in the *P. foetida* extracts were identified using LC-MS. Samples of the crude extracts were diluted in methanol and passed through a 0.2 μm PTFE syringe filter. Then, using an autosampler, 1.0 μL of the sample was injected into the column (Agilent Technologies; Poroshell 120 EC-C18; USA) size 4.6×150 mm^2 and 2.7 μm in diameter. Each sample (1 mg/mL) was eluted using a flow rate of 0.2 mL/min and a column temperature of 35 °C. Aqueous formic acid (0.1 %, v/v) (solvent A) and 0.1 % of formic acid in acetonitrile (solvent B) made up the mobile phase. The gradient program was: 5 % B, 1 - 9 min; 17 % B, 10 - 19 min; 100 % B, 20 - 26 min; and 5 % B, 27 - 33 min. A 50 - 1300 m/z scan range adjustment was made. A capillary voltage of 175.0 V for both the positive and negative modes was one of the ESI requirements. This experimental design was modified from Zhu *et al.* [17] and Phosri *et al.* [18]. Data acquisition and analysis used the MassHunter Workstation software (Agilent Technologies; Qualitative Analysis, version B.08.00; USA) and the Personal Compound Database and Library. The MS data and fragmentation profiles were compared with literature and databases such as ScienceDirect, SciFinder, and Google Scholar for *P. foetida*, with a 5 ppm error tolerance for molecular formula identification.

Tyrosinase inhibition assay

The tyrosinase inhibition assay was adapted from Cui *et al.* [19]. For this test, 80 μL of 0.1 M phosphate buffer (pH 6.8) was combined with 40 μL of the weed extract solution. After that, 40 μL of mushroom tyrosinase solution (100 units/mL) were added to the mixture, which was then incubated at 37 °C for 10 min. After incubation, 40 μL of 2.5 M L-3,4-dihydroxyphenylalanine (L-DOPA) substrate was added and the mixture was incubated again at 37 °C for 20 min. Once the incubation was complete, absorbance was immediately measured at 490 nm. Phosphate buffer was used as the negative control in place of the weed extract and kojic acid was used as the standard for comparison. The percentage of tyrosinase inhibition was calculated using Eq. (6).

$$\text{Tyrosinase inhibition (\%)} = [(A - B) - (C - D)/(A - B)]\times 100 \quad (6)$$

Where A is the absorbance of the negative control solution containing the phosphate buffer and enzyme, B is the absorbance of the solution containing only phosphate buffer (no enzyme), C is the absorbance of the sample solution with the enzyme, and D is the absorbance of the sample control solution containing the sample solution without the enzyme. The results are presented in terms of IC_{50}, which represents the minimum concentration required to inhibit 50 % of the tyrosinase enzyme activity.

Molecular docking

The tyrosinase inhibition phenolic compounds (kaempferol, chrysoeriol, and caffeoylquinic acid) were selected as ligands for the tyrosinase enzyme's inhibitory activity based on a molecular docking study. In addition, kojic acid was used as a reference compound. All 3-dimensional ligand structures were obtained from the National Institutes of Health database (https://pubchem.ncbi.nlm.nih.gov). Next, these structures were fully optimized using the Gaussian 16 software at the B3LYP/6-31G(d) level prior to the docking simulations [20]. The structure of the protein target, tyrosinase from the *Agaricus bisporus* mushroom, was retrieved from the Protein Data Bank (http://www.pdb.org), PDB ID: 2Y9X [21]. All water molecules, non-interacting ions, and a tropolone inhibitor were removed from the PDB structure, followed by adding side chains and missing hydrogen atoms using the AutoDockTools 1.5.6 program [22]. All non-polar hydrogens were merged with their corresponding carbon atoms, and Kollman and Gasteiger charges were assigned for protein and ligands, respectively [23,24]. The active site contains a number of amino acids that are partially classified as flexible, including HIS263, PHE264, MET280, VAL283, and ASN260. The grid box centered on the reference tropolone ligand was 40×40×40 grid points in the x, y, and z dimensions, with a grid spacing of 0.375 Å. These

parameters were derived from the redocking technique of the tropolone molecule as described by Asadzadeh *et al*. [25]. The AutoGrid 4.2.6 and AutoDock 4.2.6 programs were used to create energy grid maps and to search for the stable conformation of the protein-ligand complex, respectively [22]. The number of Lamarckian Genetic Algorithm runs with a maximum of 2.5×10^6 energy evaluations was set to 200, while other parameters were left at their default settings [26]. The Discovery Studio Visualizer 2024 software was used to visualize protein-ligand interactions [27]. The ligand binding affinity and inhibitory potential against protein were investigated in terms of the binding free energy ($\Delta G_{binding}$) and the inhibition constant (K_i).

Statistical analysis

For every experiment, 3 independent replications were conducted. All results were expressed as mean ± standard deviation (S.D.). For statistical comparisons, the experimental data were tested using the Shapiro-Wilk Test for normality, and using Levene's Test for homogeneity of variances. Subsequently, one-way ANOVA was conducted to compare the mean activity among different plant species. This method is suitable for comparing means between groups based on a single factor. Duncan's multiple range test is then used for post hoc analysis, with significance set at $p < 0.05$ [28].

Results and discussion

Total phenolic compound content

Analysis of the phenolic compounds in the crude leaf extracts from 3 different weeds (*U. polymorpha*, *H. suaveolens*, and *P. foetida*) revealed concentrations ranging from 4.57 - 12.99 mg/g dry extract (**Table 1**). The crude extract from *P. foetida* contained the highest phenolic content (12.99 mg/g dry extract), followed by *U. polymorpha* (5.10 mg/g dry extract) and *H. suaveolens* (4.57 mg/g dry extract), respectively. The phenolic content of the 3 extracts differed significantly at the 95 % confidence level. Ethanol as a solvent for extracting polar phenolic compounds. Polyphenols, soluble in ethanol [29,30], contribute to the antioxidant properties of these extracts. Variations in phenolic content are expected among different species and may be influenced by factors such as plant section, growing conditions, and harvest timing.

Table 1 Total phenolic compound content and antioxidant activity in crude extract of *U. polymorpha*, *H. suaveolens*, and *P. foetida*.

Sample	TPC (mg/g dry extract)	Antioxidant activity (IC_{50}; mg/mL)	
		DPPH assay	FIC assay
U. polymorpha	5.10 ± 0.05^b	0.44 ± 0.02^c	0.95 ± 0.04^b
H. suaveolens	4.57 ± 0.09^a	0.07 ± 0.00^a	12.80 ± 0.07^c
P. foetida	12.99 ± 0.10^c	0.23 ± 0.01^b	0.35 ± 0.02^a
Gallic acid	-	0.01 ± 0.00	-
EDTA	-	-	0.09 ± 0.02

Values are displayed as mean ± S.D. values (n = 3). Lowercase superscripts within same column are significantly different at $p < 0.05$.

Antioxidant activity

The antioxidant activity of the crude extracts was assessed using the DPPH assay, with IC_{50} values ranging from 0.07 to 0.44 mg/mL (**Table 1**). Notably, *H. suaveolens* showed the highest DPPH radical scavenging capacity with IC_{50} values 0.07 mg/mL. Previous studies support this, indicating that plants within the Suaveolens species effectively scavenge DPPH radicals [31,32]. The presence of phenolic compounds in these extracts likely contributes to their antioxidant action, as they stabilize free radicals by donating hydrogen atoms [33]. Gallic acid, a phenolic compound commonly used in cosmetics, has excellent free radical scavenging activity. In the current study,

gallic acid had an IC_{50} value of 0.01 mg/mL (**Table 1**). The crude extract from *H. suaveolens* also had notable free radical scavenging activity, though it was 7 times weaker than that of gallic acid. However, the strong antioxidant activity of *H. suaveolens* may be attributed not only to its phenolic compounds but also to its terpenoids and alkaloids, which can also exhibit antioxidant properties.

The FIC method was used to assess the metal chelating ability, with the findings showing the IC_{50} value range was 0.35 - 12.80 mg/mL (**Table 1**). The crude extracts of *P. foetida* had the highest metal chelating capability, suggesting the presence of ortho-hydroxyphenyl structures or an ortho-hydroxyl-oxo group in its phenolic compounds [34]. Caffeoylquinic acid, orientin, and chrysin 7-glucoside have both structures, while kaempferol and chryoeriol have ortho-hydroxyphenyl structures (**Table 4**). While EDTA served as standard with an IC_{50} of 0.09 mg/mL (**Table 1**), the effectiveness of *P. foetida* was approximately 4 times lower. Commonly, EDTA is used in moisturizers, skin care products, and cleansers to prevent the degradation of cosmetic formulations.

Antibacterial activity

The antibacterial efficacy of the extracts was evaluated through broth dilution assays, revealing MIC values of 1.00 mg/mL for *U. polymorpha* and *H. suaveolens*, and 0.50 mg/mL for *P. foetida*, with reductions of approximately 90 % in *B. subtilis* bacterial populations (**Table 2**). Clindamycin has a MIC of 0.004 mg/mL against *B. subtilis* [35]. Compared to clindamycin, the *P. foetida* extract exhibits relatively weak antibacterial activity. The phenolic content likely plays a significant role in this antibacterial activity, aligning with existing literature that highlights the importance of phenolic compounds in inhibiting bacterial growth [3,36,37]. Furthermore, it has been reported that some weed extracts have the capacity to inhibit the growth of *E. coli* [7]. In addition, the leaf extract of *P. foetida* was reported to inhibit *Streptococcus pyogenes*, with a MIC of 0.104 mg/mL and an MBC exceeding 0.25 mg/mL [38].

Table 2 Antibacterial activity in crude extract of *U. polymorpha*, *H. suaveolens*, and *P. foetida*.

Organism	Concentration of crude extract (mg/mL)	Bacterial reduction (%)		
		U. polymorpha	***H. suaveolens***	***P. foetida***
S. aureus	1.00	74.05 ± 3.12^{i}	72.87 ± 3.58^{i}	83.91 ± 4.30^{ii}
	0.50	$75.19 \pm 3.76^{\lozenge}$	$73.54 \pm 2.61^{\lozenge}$	$77.13 \pm 2.61^{\lozenge}$
	0.25	$63.34 \pm 2.84^{*}$	$70.56 \pm 2.92^{**}$	$70.55 \pm 2.65^{**}$
S. epidermidis	1.00	$79.87 \pm 2.35^{\#}$	$84.82 \pm 0.45^{\#\#}$	$87.38 \pm 4.53^{\#\#\#}$
	0.50	$70.60 \pm 3.57^{!}$	$84.20 \pm 3.27^{!!}$	$83.56 \pm 0.96^{!!}$
	0.25	$70.21 \pm 1.16^{\circ}$	$84.19 \pm 2.21^{\circ\circ}$	$82.85 \pm 0.96^{\circ\circ}$
E. coli	1.00	$74.06 \pm 3.12^{///}$	$42.13 \pm 0.74^{//}$	$36.12 \pm 1.59^{/}$
	0.50	51.34 ± 0.78^{xx}	26.06 ± 0.86^{x}	24.98 ± 0.59^{x}
	0.25	$44.03 \pm 1.73^{\wedge\wedge\wedge}$	$24.78 \pm 1.28^{\wedge\wedge}$	$9.12 \pm 0.52^{\wedge}$
B. subtilis	1.00	$95.98 \pm 1.92^{\tilde{}}$	$93.86 \pm 3.15^{\tilde{}}$	$95.31 \pm 2.24^{\tilde{}}$
	0.50	$89.40 \pm 0.80^{\tilde{\tilde{}}}$	$80.46 \pm 1.04^{\tilde{}}$	$90.02 \pm 1.16^{\tilde{\tilde{}}}$
	0.25	$88.64 \pm 2.33^{\square\square}$	$74.08 \pm 2.69^{\square}$	$84.55 \pm 4.10^{\square\square}$
B. cereus	1.00	$78.76 \pm 3.00^{\Theta\Theta}$	$81.47 \pm 1.20^{\Theta\Theta\Theta}$	$75.11 \pm 1.67^{\Theta}$
	0.50	$64.52 \pm 0.45^{\delta\delta}$	$76.29 \pm 2.15^{\delta\delta\delta}$	$58.04 \pm 0.92^{\delta}$
	0.25	$56.54 \pm 2.13^{\cdot}$	$69.37 \pm 1.08^{\cdot\cdot}$	$56.13 \pm 1.19^{\cdot}$

Values are displayed as mean ± S.D. (n = 3). Symbols within the same row are significant differences at $p < 0.05$.

Effects of extracts on morphological microbial cells

Using SEM, morphological changes in *B. subtilis* exposed to the extracts were observed, demonstrating significant alterations in cell membrane integrity (**Figure 3**). The extracts led to a wrinkled or leaky appearance in bacterial cells, particularly evident with *P. foetida*. This suggests that phenolic compounds may disrupt bacterial membranes, consistent with studies showing that these compounds can impair bacterial cell wall stability [39,40].

In the examined strain of *B. subtilis*, the polysaccharide present in the bacterial cell wall might undergo structural changes due to chemical interactions with phenolic molecules. Consequently, the bacterial cell experiences deformation as the cell wall loses its equilibrium. Based on the results in the current study, the most substantial wrinkling of *B. subtilis* was induced by the *P. foetida* leaf extract, which had the highest phenolic compound content ($p < 0.05$).

The antibacterial properties of plant phenolic compounds arise from their ability to inhibit vital enzymes and disrupt or impair the functionality of genetic material and interact with bacterial cell membranes [39,40].

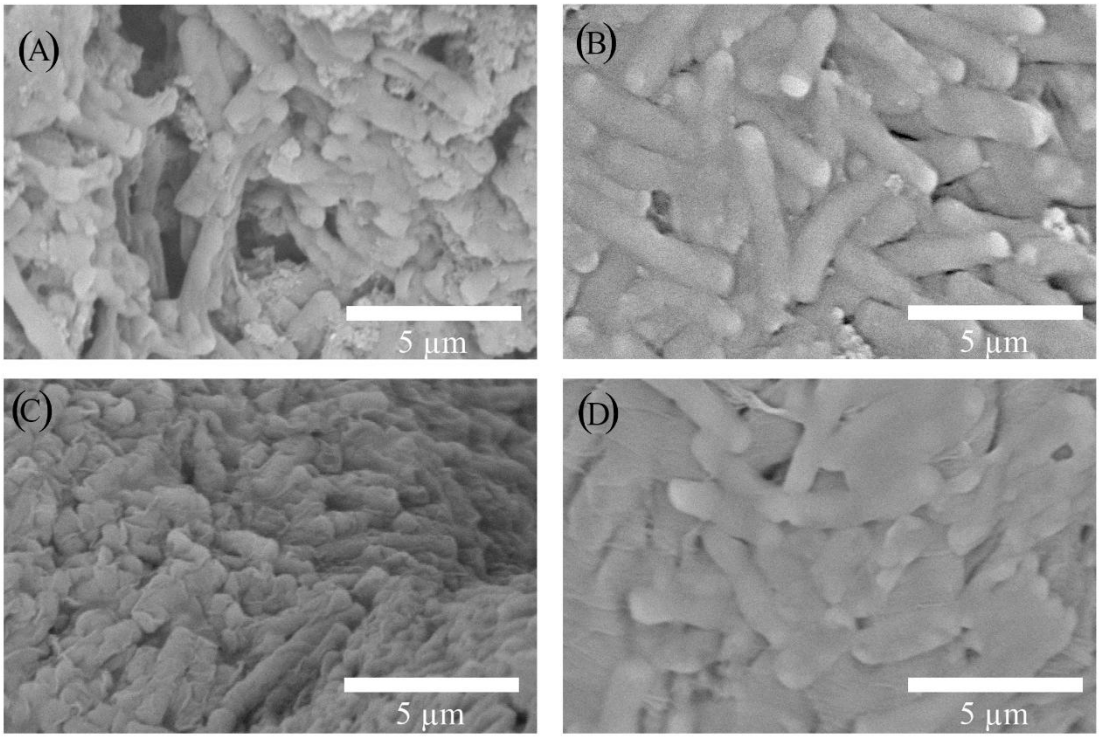

Figure 3 SEM images of *B. subtilis* after incubation with (A) *U. polymorpha*, (B) *H. suaveolens*, (C) *P. foetida* and (D) control cells were treated without any sample for 2 h.

Cytotoxicity assay

Cytotoxicity assays revealed that extracts from *U. polymorpha*, *H. suaveolens*, and *P. foetida* were non-toxic to 3T3 fibroblast cells, Vero epithelial cells, and RAW 264.7 macrophages across various concentrations (**Figure 4**). The survival rate of over 80 % of cells in the current experiment indicated that the extract was not harmful to them. Closely related, these weed species have also been reported to inhibit the following disease-associated cell lines; The extracts of *Urceola huaitingii* stem can inhibit gastric cancer cell growth [41]. The ethanol and aqueous extracts of *H. suaveolens* leaves selectively decreased human leukemia T cells (Jurkat cells) without affecting normal peripheral blood mononuclear cells [42].

Additionally, the essential oil extracted from the

leaves of *H. suaveolens* was toxic to the insect pest *Drosophila melanogaster* [43]. *P. foetida* leaf extract was reported to be non-toxic to experimental rats when administered at a dose of 1,600 mg/kg/day [44]. Furthermore, the leaf contained vitexin, a compound with anti-inflammatory properties [44]. The results suggest that the extracts promote cellular health and could be further explored for applications in medicine and dietary supplements.

Phytochemicals identified using LC-MS

P. foetida leaf extract was used in the current phytochemical studies due to its potent antibacterial and antioxidant qualities, as well as having a phenolic concentration twice as high as that of extracts from *U. polymorpha* or *H. suaveolens* (**Table 1**). The leaf extract from *P. foetida* was evaluated for its chemical composition using both positive (**Table 3**) and negative (**Table 4**) ion modes of LC-MS. Flavonoids are the most prevalent class of phenolic chemicals, including: Callistephin, orientin, isoscoparin or scoparin, Chrysin-7-glucoside, kaempferol and chrysoeriol, as shown in **Table 4**. These findings were consistent with other research on the occurrence of secondary metabolites in *Passiflora* species, specifically flavonoids [45]. Additionally, 3 fatty acid lactones; passifetilactone A, passifetilactone B, and passifetilactone D were identified in the leaf extract of *P. foetida*. Ponsuwan *et al*. [45] reported the presence of 4 types of fatty acid lactones (passifetilactones A - D) in the fruits and flowers of *P. foetida.* Among these, passifetilactone B significantly suppressed KKU-055 cancer cells.

Table 3 Phytochemicals identified based on positive-ion LC-MS data in *P. foetida*.

No	RT (min)	Mass	m/z (expected)	Abundance	Chemical formula	Error (ppm)	Putatively identification	Match score
Alkaloid								
1	18.447	212.0940	235.0848	2157	$C_{13}H_{12}N_2O$	–4.34	Harmine	83.80
Fatty acid ester								
2	21.211	340.2992	358.3332	37204	$C_{21}H_{40}O_3$	4.37	Carbonic acid octadecylvinylester	92.00
Fatty acid								
3	21.971	280.2402	281.2469	3162	$C_{18}H_{32}O_2$	0.04	9, 12- Linoleic acid	76.28
Volatile compounds								
4	23.013	204.1880	222.2219	385	$C_{15}H_{24}$	1.21	α-Humulene	70.78
Triterpenoid glycoside								
5	23.989	710.4254	728.4600	14396	$C_{38}H_{62}O_{12}$	1.78	(31R)-31-O-Methylpassiflorine	72.83

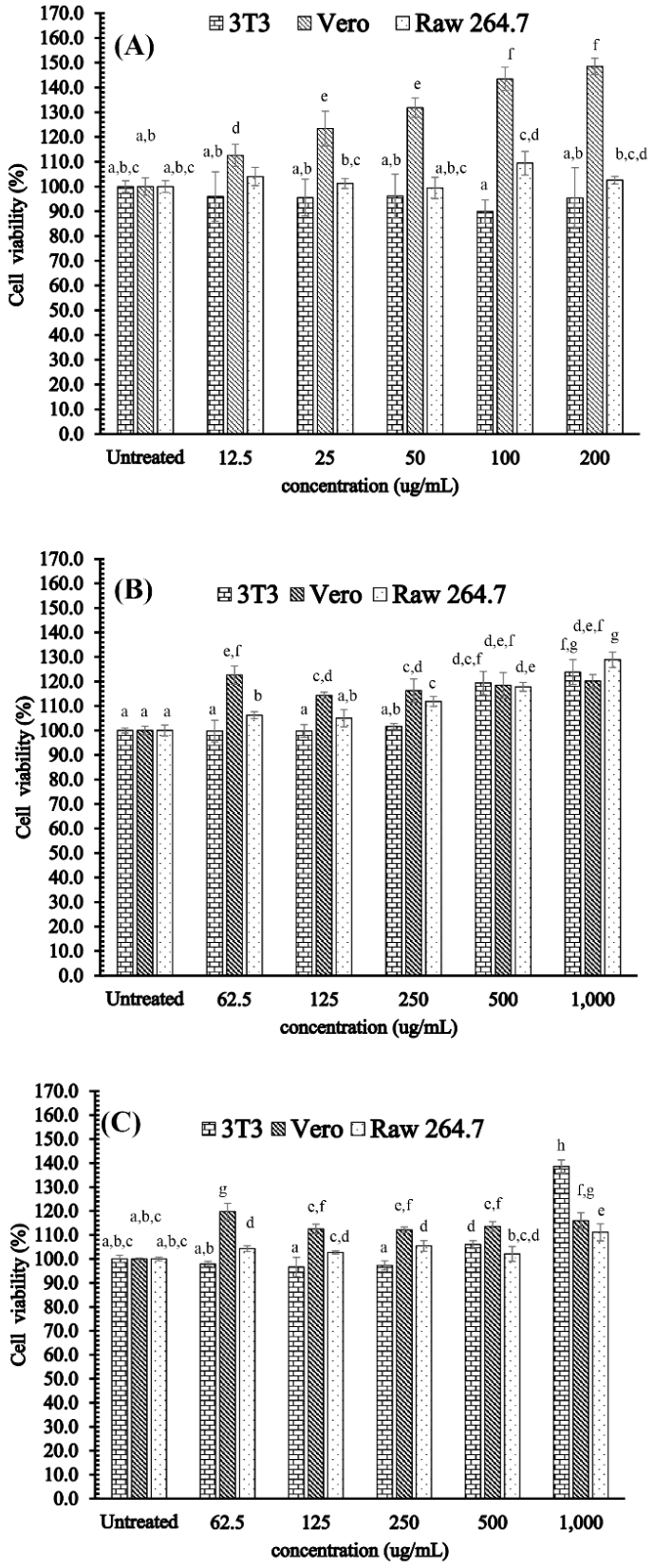

Figure 4 Cytotoxicity activities of (A) *U. polymorpha*, (B) *H. suaveolens* and (C) *P. foetida* leave extracts with different concentrations determined based on MTT assay. Fibroblast cell (3T3), African green monkey epithelial cells (Vero), and murine macrophage cell lines (RAW 264.7) were used for assaying. In each graph, different letters above the bars represent significant differences at $p < 0.05$. Error bars indicate ± S.D. (n = 3).

Table 4 Phytochemicals identified base on negative-ion LC-MS data in *P. foetida*.

No	RT (min)	Mass	m/z (expected)	Abundance	Chemical formula	Error (ppm)	Putatively identification	Match score
Flavonoids								
1	15.148	433.1135	432.1063	19490	$C_{21}H_{21}O_{10}$	0.15	Callistephin	81.52
2	16.094	448.1004	447.0929	9062	$C_{21}H_{20}O_{11}$	–0.29	Orientin	96.35
3	16.834	462.1159	461.1084	5036	$C_{22}H_{22}O_{11}$	–0.66	Isoscoparin or Scoparin	92.78
4	16.834	416.1102	461.1084	5036	$C_{21}H_{20}O_{9}$	–1.33	Chrysin 7-glucoside	92.78
5	17.795	286.047	285.0399	80882	$C_{15}H_{10}O_{6}$	–2.43	Kaempferol	94.12
6	18.439	300.0627	299.0556	89439	$C_{16}H_{12}O_{6}$	–2.18	Chrysoeriol	95.54
Triterpenoid								
7	20.394	860.4768	919.4892	530	$C_{43}H_{72}O_{17}$	–0.12	Cyclopassifloside IX	87.69
8	23.84	504.3450	503.3376	34835	$C_{30}H_{48}O_{6}$	–0.23	Cyclopassifloic acid D	99.3
Fatty acids								
9	18.64	242.2243	287.2225	39319	$C_{15}H_{30}O_{2}$	–1.36	Pentadecanoic acid	97.99
10	21.984	258.2192	257.2119	26455	$C_{15}H_{30}O_{3}$	–1.21	15-Hydroxypentadecanoic acid	99.36
11	22.904	278.2251	277.2178	48502	$C_{18}H_{30}O_{2}$	1.93	Linolenic acid	98.21
12	23.775	280.2396	279.2324	23804	$C_{18}H_{32}O_{2}$	–2.22	9, 12- Linoleic acid	98.19
13	23.923	284.2726	343.2865	434649	$C_{18}H_{36}O_{2}$	3.91	Stearic acid	95.26
14	25.032	256.2404	255.2330	10382	$C_{16}H_{32}O_{2}$	0.57	Palmitic acid	99.32
Fatty aldehydes								
15	21.259	184.1824	243.1962	7426	$C_{12}H_{24}O$	–1.95	Methyl nonyl acetaldehyde	97.94
16	21.984	212.2139	271.2279	15331	$C_{14}H_{28}O$	–0.70	Tetradecanal	99.77
17	23.018	238.2295	297.2432	24435	$C_{16}H_{30}O$	–0.76	cis-11-Hexadecenal	99.19
18	23.31	264.2451	323.2591	10922	$C_{18}H_{32}O$	–0.63	9,17-Octadecadienal, (Z)-	84.61
19	23.502	252.2450	297.2432	13508	$C_{17}H_{32}O$	–1.2	Trogodermal	98.5
20	23.943	266.2614	325.2754	1123	$C_{18}H_{34}O$	1.51	10-Octadecenal	86.94
Fatty acid esters								
21	22.223	320.2719	365.2705	5942	$C_{21}H_{36}O_{2}$	1.05	Eicosatrienoic acid methyl ester	92.46
22	22.406	340.2986	385.2968	234302	$C_{21}H_{40}O_{3}$	2.44	Carbonic acid octadecylvinylester	97.88
23	22.703	296.2716	341.2698	259111	$C_{19}H_{36}O_{2}$	0.12	11-Octadecenoic acid, methyl ester	98.63
24	22.703	282.2559	341.2698	259111	$C_{18}H_{34}O_{2}$	0.12	E-11-Hexadecenoic acid, ethyl ester	98.63
25	22.949	308.2722	367.2857	13815	$C_{20}H_{36}O_{2}$	2.20	Ethyllinoleate	80.43
26	24.055	310.2866	369.3007	25782	$C_{20}H_{38}O_{2}$	–1.98	Ethyl Oleate	97.27
27	26.097	312.3028	371.3166	7028	$C_{20}H_{40}O_{2}$	–0.17	Ethyl Stearate	98.14
Fatty acid lactones								
28	22.223	306.2563	365.2705	5942	$C_{20}H_{34}O_{2}$	1.52	Passifetilactone A	92.46
29	24.66	324.2655	369.2637	10054	$C_{20}H_{36}O_{3}$	–2.93	Passifetilactone B	94.64
30	24.758	352.2976	351.2906	1082	$C_{22}H_{40}O_{3}$	–0.43	Passifetilactone D	94.95
Fatty acid amide								
31	23.383	281.2730	326.2710	4526	$C_{18}H_{35}NO$	3.98	Oleamide	96.23

No	RT (min)	Mass	m/z (expected)	Abundance	Chemical formula	Error (ppm)	Putatively identification	Match score
Volatile oil compounds								
32	17.398	240.1724	299.1866	1388	$C_{14}H_{24}O_3$	–0.45	Oxacyclotetradecane-2,11-dione, 13-methyl-	80.66
33	17.52	104.0623	103.055	1520	C_8H_8	–2.65	Styrene	83.89
34	21.304	224.2497	283.2637	1395	$C_{16}H_{32}$	–2.94	7-Hexadecene, (Z)-	81.25
35	21.984	222.1979	267.1961	1913	$C_{15}H_{26}O$	–2.25	7a-Isopropenyl-4,5-dimethyloctahydroniden-4ylmethanol	84.73
36	22.304	210.1986	269.2124	8327	$C_{14}H_{26}O$	0.97	9,12-Tetradecadien-1-ol, (Z,E)-	86.3
37	22.607	280.3122	339.3258	348	$C_{20}H_{40}$	–2.90	Trans-3-eicosene	81.01
38	23.923	240.2816	299.2956	531	$C_{17}H_{36}$	–0.44	Heptadecane	80.34
39	23.943	254.2971	299.2956	406	$C_{18}H_{38}$	–1.08	Octadecane	80.77
40	25.032	196.2192	255.2330	10382	$C_{14}H_{28}$	0.75	Cyclotetradecane	99.32
Miscellaneous compounds								
41	4.641	354.0952	413.1091	9037	$C_{16}H_{18}O_9$	0.20	Caffeoylquinic acid	99.4
42	17.52	196.1092	241.108	1491	$C_{11}H_{16}O_3$	–3.94	Isololiolide	83.85
43	19.909	250.1935	309.207	371585	$C_{16}H_{26}O_2$	0.90	Acetic acid, 1-methyl-3-(2,2,6-trimethylbicyclo [4.1.0]hept-1-yl)-propenyl ester	90.6
44	20.235	108.0576	167.0715	4730	C_7H_8O	0.74	Benzyl alcohol	86.24
45	21.984	168.1877	213.1861	1871	$C_{12}H_{24}$	–0.74	1-Dodecene	82.25
46	22.904	334.2148	333.2075	2309	$C_{20}H_{30}O_4$	1.22	Phthalic acid, isobutyl octyl ester	84.39

Tyrosinase inhibitory activity

Tyrosinase is a crucial enzyme in the production of melanin. Melanin plays a crucial role in protecting the skin from UV radiation. However, overproduction of melanin can lead to skin conditions such as dark spots, freckles, and melasma. Thus, tyrosinase inhibitors have interesting applications in the beauty and pharmaceutical sectors. The crude extract from *P. foetida* leaves was selected for this experiment, due to its high phenolic content. The anti-tyrosinase activity of *P. foetida* leaves had an IC_{50} value of 64.04 mg/mL (**Table 5**). While tyrosinase inhibitory effect of this crude extract is lower than kojic acid (IC_{50} 0.12 mg/mL), the presence of flavonoids such as kaempferol and chrysoeriol suggests potential for development as a natural tyrosinase inhibitor. Molecular docking studies indicated that these flavonoids could effectively bind to tyrosinase, supporting their inhibitory potential (**Table 6**). However, other substances in the extract might negate or offset its overall enzyme inhibiting effectiveness.

Table 5 Tyrosinase inhibition by crude extract of *P. foetida*.

Sample	IC_{50} (mg/mL)
Crude extract of *P. foetida*	64.04 ± 1.49
Kojic acid	0.12 ± 0.00

Values in the table are displayed as mean ± S.D. values (n = 3).

Molecular docking

The crude extract from the *P. foetida* leaves was selected for its high phenolic content, which has been linked to antioxidant properties and tyrosinase inhibition. The LC-MS analysis identified flavonoids, particularly kaempferol and chrysoeriol, with high abundance and high match scores. These compounds were chosen for molecular docking studies due to their ability to bind copper ions at the enzyme's active site, potentially inhibiting its function. Kaempferol is known to inhibit tyrosinase [46], and chrysoeriol is hypothesized to have similar effects. Additionally, caffeoylquinic acid, a miscellaneous compound with a high match score (99.4), was included for further molecular docking studies. The molecular docking results are shown in **Table 6**. Only kojic acid, kaempferol, and chrysoeriol had persistent docking conformations inside the active site, while caffeoylquinic acid lacked a distinct binding site for the enzyme, presumably owing to structural incompatibility. The 3 docked compounds displayed major interactions with key amino acid residues via hydrogen bonds, hydrophobic interactions, and van der Waals forces, enhancing their binding affinity and inhibitory their efficacy against tyrosinase (**Figure 5**). Notably, compounds with elevated binding affinity scores have enhanced inhibitory potential, indicating effectiveness in tyrosinase inhibition. Kojic acid, used as a benchmark for tyrosinase inhibition research in the laboratory, had 2 docking conformations inside the active site, with marginally distinct orientations. Both conformations yielded a binding free energy ranging from –5.82 to –4.91 kcal/mol, resulting in an inhibition constant in the tens to hundreds micromolar range. Kojic acid is a small molecule composed of several atoms that can serve as both hydrogen donors and acceptors.

Consequently, it may readily establish hydrogen bonds with amino acids at the active site, in particular, the amino acid residues HIS259, ASN260, HIS263, and MET280, which are located deep inside the tiny active site pocket, shown in **Figure 5(A)**. Kaempferol, the most interesting phenolic compound present in weeds, has values for $\Delta G_{binding}$ of –7.76 kcal/mol and of K_i of around 2 μM. The molecular docking results revealed a very high docking score of 62.5 %, indicating that the ligand's binding location was highly specific to the shape of the tyrosinase active site. The result was consistent with the findings of Farasat *et al*. [47], who demonstrated that kaempferol exhibited selectivity for tyrosinase.

Kaempferol is a flavonoid consisting of 4 hydroxyl groups that may form hydrogen bonds, making the molecule too bulky to penetrate the active site effectively. The docked structure indicated that the phenyl group of the molecule reorients inward, forming hydrogen bonds with HIS259, HIS263, and HIS296, as seen in **Figure 5(B)**. The docking results of chrysoeriol revealed 3 possible docked structures with slightly different orientations (**Figure 5(C)**). As can be seen in **Table 6**, chrysoeriol has comparable binding affinity to kaempferol, probably attributable to their similar structures. The orientation of chrysoeriol in the 3 docking conformations is contrary to that of kaempferol. The benzopyran moiety was oriented inward inside the deep cavity of the active site. This compound can form hydrogen bonds with amino acid residues, including GLU256, ASN260, HIS263, and VAL283.

Notably, a single compound, including kaempferol and chrysoeriol, demonstrated superior tyrosinase inhibitory efficacy compared to kojic acid, whereas the experimental analysis of the crude extract of *P. foetida* produced a higher IC_{50} value than the standard kojic acid (**Table 5**), probably due to the interference of multiple compounds in the crude extract with the binding of active compounds to the active site. This indicates that their separation may result in effective tyrosinase inhibitors. Molecular docking also suggests that these flavonoids could be promising for further development in tyrosinase inhibition.

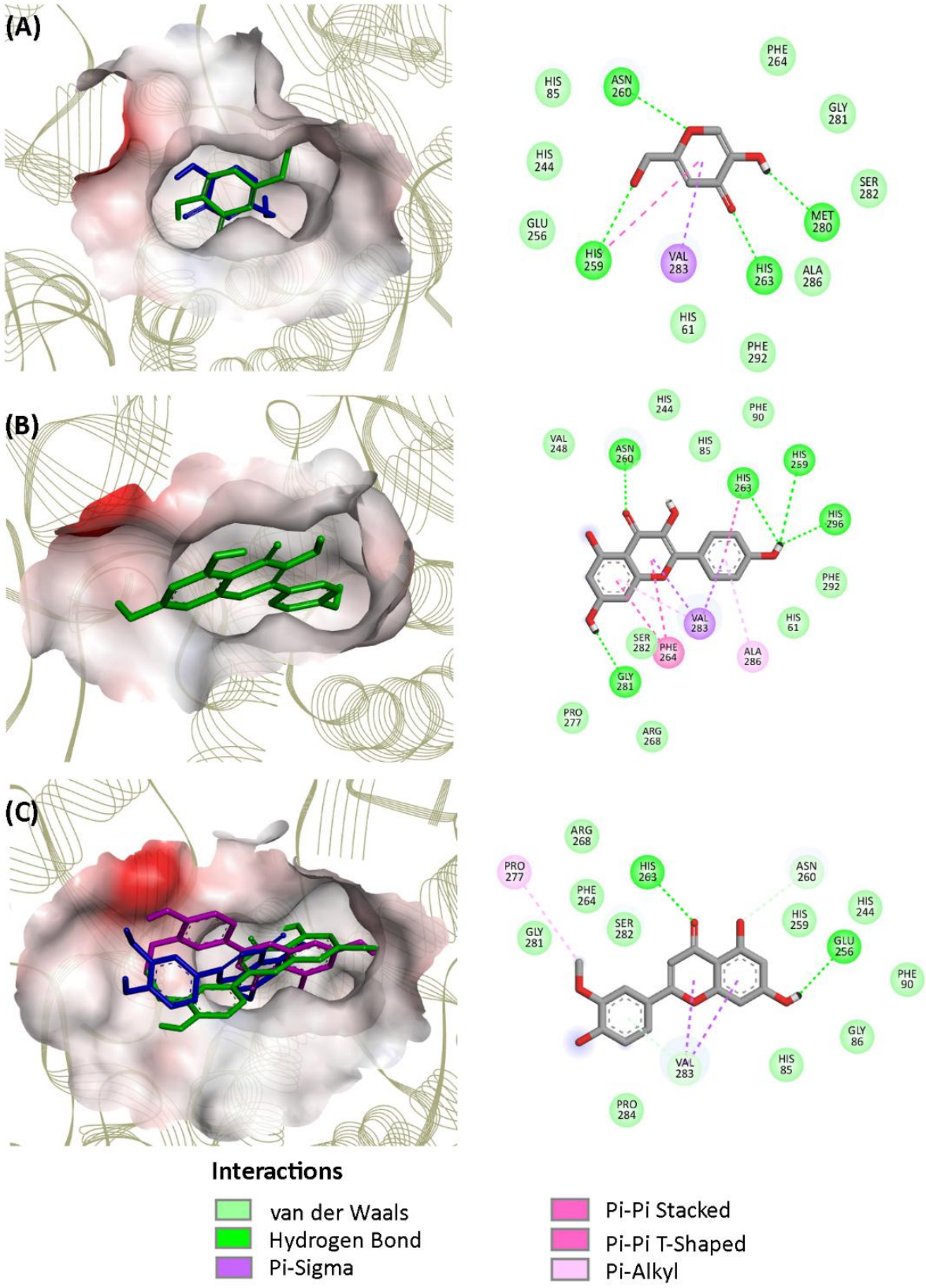

Figure 5 Overview docking conformations and molecular interactions of (A) kojic acid, (B) kaempferol, and (C) chrysoeriol, within the tyrosinase active site. In 3D structures, green, blue, and magenta refer to docking conformations 1, 2, and 3, respectively. The 2D structures show only docking conformation 1, yielding the lowest binding free energy.

Table 6 Molecular docking results of the bioactive compounds with tyrosinase enzyme.

Compound	Docking conformation	$\Delta G_{binding}$ (kcal/mol)	K_i (mM)	Binding interactions	
				H-bond	Hydrophobic
Kojic acid	1	–5.82	54.06	HIS259, ASN260, HIS263, MET280	HIS259, VAL283
	2	–4.91	250.85	HIS259, ASN260, HIS263, HIS296	HIS263, ALA286
Kaempferol	1	–7.76	2.05	HIS259, ASN260, HIS263, GLY281, HIS296	HIS263, PHE264, VAL283, ALA286
Chrysoeriol	1	–8.12	1.12	GLU256, ASN260, HIS263, VAL283	PRO277, AL283
	2	–7.95	1.48	ASN260, ARG268, MET280, VAL283	PHE264, LEU275, PRO277, VAL283, ALA286
	3	–7.38	3.88	GLU256, ASN260, HIS263, ARG268, GLY281	PHE264, PRO277, VAL283

Conclusions

The study examined various weed extracts, all of which displayed good biological properties. Notably, the extract from the leaves of *P. foetida*, which had the highest concentration of phenolic substances, antioxidants, and antibacterial agents and was non-toxic to normal cells. Additionally, it inhibited tyrosinase enzymes. Based on the molecular docking studies, kaempferol and chrysoeriol were identified as potential inhibitors of tyrosinase, demonstrating considerable selectivity for the enzyme's active site. This discovery suggests that this weed could serve as a valuable source of natural antibacterial and antioxidant compounds. In addition to their bioactive properties, weeds contribute to ecosystem stability by enriching soil, preventing erosion, and supporting biodiversity by serving as food and shelter for various organisms. Recognizing these benefits underscores the importance of utilizing weeds sustainably rather than simply eliminating them. Innovative use of weed extracts maximizes their benefits while avoiding mere elimination. Furthermore, eco-conscious harvesting during peak seasons with proper intervals ensures sustainable propagation while maintaining their role in ecological balance. Moreover, future *in vivo* studies on active ingredients may be undertaken to deepen understanding of their mechanisms and facilitate their effective application. By taking these steps, the full potential of weed extracts can be sustainable and responsibly harnessed.

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Declaration of Generative AI in Scientific Writing

During the preparation of this manuscript, the authors used Microsoft 365 Copilot solely for grammar and spelling correction. The tool was employed under human oversight and did not contribute to the generation of scientific content, data analysis, or interpretation. All outputs were carefully reviewed and edited by the authors, who take full responsibility for the final version of the manuscript.

CRediT author statement

All Authors: Conceptualization; Methodology; Investigation. **Sutthidech Preecharram, Jinda Jandaruang**: Writing - Original draft. Nattawee Poomsuk: Software; Validation. **All Authors**: Writing - Reviewing and Editing.

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