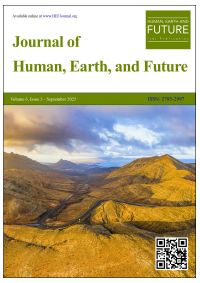
Available online at www.HEFJournal.org

ISSN: 2785-2997

Journal of
Human, Earth, and Future

Vol. 6, No. 3, September, 2025



Antioxidation, Antimicrobial, and Cytotoxic Activities of Some Mangrove Plants

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Received 06 January 2025; Revised 26 May 2025; Accepted 15 June 2025; Published 01 September 2025

Abstract

Twenty-three samples of mangrove plants were refluxed with methanol, then their activities were evaluated. Surprisingly, sample 6 (%SC_{50} = 5.00 ± 0.08 µg/mL) had the best antioxidation activity among candidates and L-ascorbic acid. Sample 19 was the second, with %SC_{50} = 5.15 ± 0.15 µg/mL. Both presented the availability of flavonoids and tannins, which was confirmed by phytochemical screening. The antimicrobial assay was done parallelly with the DPPH-free radical scavenging assay. Sample 19 exhibited the highest bacteriostatic activities against *S. aureus*, *A. baumannii*, *P. aeruginosa* (MIC = 62.50 µg/mL), and *E. faecalis* (MIC = 500 µg/mL) due to the presence of terpenes. In the MTT assay, it was found that sample 19 displayed specific toxicity against KB (%IC50 = 18.51 ± 5.49 µg/mL) and HeLa (%IC50 = 160.68 ± 39.36 µg/mL). Samples 2, 4, and 20 also demonstrated selective toxicity against cancer cells. This is the first report of sample 19, which is the most effective and potent cytotoxic agent against KB, and sample 4, which is the specific and most potent cytotoxic agent against HeLa. This is the first time mangrove plants have been evaluated for their potential to be alternative natural sources of medicine.

Keywords: Mangrove Forest; Antioxidant; Antimicrobial Activity; Cytotoxic Activity.

1. Introduction

Nakhon Si Thammarat Province, located along the Gulf of Thailand, boasts a 32-kilometres-long coastline that spans districts such as Khanom, Thasala, Muang Nakhon Si Thammarat, and Pakphanang [1]. This area is home to diverse mangrove ecosystems comprising freshwater, brackish water, and saltwater mangroves, contributing to its abundant natural resources. Traditionally, Thai medicine has utilized various mangrove plants for therapeutic purposes [2]. For example, mangrove-derived remedies have been used as febrifuges (e.g., *Rhizophora apiculata*, *Tribulus terrestris*, *Glycosmis pentaphylla*), treatments for diarrhea (e.g., *Xylocarpus granatum*, *Rhodomyrtus tomentosa*, *Sonneratia caseolaris*), wound healing (e.g., *Lumnitzera racemosa*, *Wedelia biflora*, *Ipomoea pes-caprae*), and anticancer therapies (e.g., *Acanthus ebracteatus*, *Catunaregam spathulifolia*, *Hedyotis corymbose*), though scientific validation for these applications is still required [3]. In addition to medicinal uses, mangrove resources have historically supported other

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http://dx.doi.org/10.28991/HEF-2025-06-03-04

industries. For instance, the stems of *Rhizophora apiculata* are used to produce charcoal, its leaves are utilized as natural dyes for tie-dye fabrics, and the leaves of *Rhizophora mucronata* are employed in food preparation [4]. However, with advancements in medical accessibility and faster healthcare delivery, the reliance on mangrove plants for health benefits has declined [5]. This research explores the potential of mangrove plant extracts as sources of biologically active compounds. During fieldwork, 17 mangrove plant species were collected from Nakhon Si Thammarat Province, resulting in 23 samples. These samples were subjected to preliminary biological activity assays, including antioxidant, antimicrobial, and anticancer evaluations. The findings are expected to identify novel medicinal sources, contributing to advancements in medical and pharmaceutical applications.

2. Material and Methods

Between April and July 2023, a diverse set of 23 plant samples representing 17 medicinal species was systematically collected from the mangrove ecosystems of Nakhon Si Thammarat Province. These samples were sourced from various districts, including Khanom (samples 7–9, 11–14, 17–18, 22–23), Thasala (samples 4–5), Muang Nakhon Si Thammarat (sample 3), and Pakphanang (samples 1–2, 6, 10, 15–16, 19–21). Each collection was carefully documented, with voucher specimens authenticated and archived at Walailak Botanical Park, Walailak University, to ensure traceability and support future botanical studies. The collected samples were categorized into specific plant parts, such as leaves, stems, roots, seeds, and fruit peels, to facilitate targeted extraction of bioactive compounds. These parts were dried at 45 °C in a hot-air oven, preserving their phytochemical integrity. After drying, the materials were finely milled into powders to increase surface area for efficient extraction. The powdered plant materials were subjected to methanolic extraction using a reflux method, conducted in three cycles (1.0 L of methanol per cycle for 3 hours each) to maximize yield. The resulting methanolic extracts were combined, filtered to remove particulate matter, and concentrated under reduced pressure to obtain crude extracts free of volatile solvents. These crude extracts provided a concentrated source of bioactive compounds and served as the foundation for subsequent chemical and biological analyses. This meticulous approach ensured the preservation of plant biodiversity while laying the groundwork for pharmacological exploration.

2.1. Phytochemical Screening

2.1.1. Test for Tannins

The presence of tannins in crude methanolic extracts of the sample was determined by the methods as mentioned in the previous reports. This reaction produced distinct color changes, allowing for the differentiation between tannin types: a greenish-black or turquoise precipitate indicated the presence of condensed tannins. In contrast, a deep blue precipitate was characteristic of hydrolysable tannins. These methods provide a straightforward yet practical approach to classifying tannins and elucidating their structural diversity within plant-derived extracts [5].

2.1.2. Tests for Steroids and Terpenoids (Liebermann–Burchard Test)

To detect the presence of steroids and terpenoids in methanolic extracts, the Liebermann-Burchard test was applied. This reaction yielded distinctive color changes: a green to blue-green hue indicated the presence of steroids, whereas a purple-red or magenta coloration confirmed the presence of terpenoids. This method provides a reliable and visually interpretable approach for qualitatively analyzing these bioactive compounds [6].

2.1.3. Test for Alkaloids

To identify alkaloids in the crude methanolic extracts, a portion of each sample was subjected to acid hydrolysis then reacted with Dragendorff's reagent, a widely used chemical indicator for alkaloid detection. The appearance of an orange-red amorphous precipitate confirmed the presence of alkaloid compounds in the samples. This straightforward assay leverages the reactivity of alkaloids with the reagent to provide a visual confirmation of their presence [7].

2.1.4. Test for Flavonoids

A qualitative test was conducted to determine the presence of specific flavonoid subclasses in methanolic leaf extracts. The ammonium hydroxide test solution was induced to distinct color changes and precipitate formation, indicating different flavonoid types. The emergence of a yellow precipitate signified the presence of flavones or flavonols, while an orange-red hue indicated flavanones. In contrast, the appearance of a magenta-colored solution suggested the presence of chalcones or aurones. These colorimetric changes provide a simple yet effective approach to preliminarily classify flavonoids within complex plant extracts [8].

2.2. Biological Evaluation

2.2.1. Antioxidation Assay

The antioxidant capacity of the crude methanolic extracts was evaluated using a DPPH free-radical-scavenging assay. This method provided a reliable measure of the extracts' ability to neutralize free radicals by donating hydrogen atoms

or electrons. To ensure precision, the preparation of the test solutions, positive controls, and experimental samples incorporated slight modifications to enhance the reproducibility and relevance of the protocol, adapting elements from the approach outlined initially by Braca et al. (2001) [9]. These adjustments aimed to optimize the experimental conditions while maintaining consistency with established methodologies. L-ascorbic acid was employed as the positive control. Similarly, all sample extracts were dissolved and serially diluted with methanol to achieve final concentrations. For the assay, 100 µL of each positive control or sample solution at varying concentrations was mixed with 100 µL of DPPH solution. Establishing a baseline for the DPPH assay, the control sample was prepared by combining 100 µL of DPPH solution with an equal volume of methanol. In comparison, the blank control consisted of 200 µL of pure methanol to account for background absorbance. A blank sample was done by mixing the sample and methanol, 1: 1. All mixtures were incubated in darkness for 30 minutes to prevent light-induced reactions. Absorbance readings were subsequently recorded at 517 nm using a microplate reader. These readings formed the basis for calculating $\%SC_{50}$ values, which quantify the sample concentration required to achieve a 50% reduction in DPPH radical activity [9].

$$\%SC = \frac{[Ab_{control} - Ab_{control\ blank}] - [Ab_{sample} - Ab_{sample\ blank}]}{[Ab_{control} - Ab_{control\ blank}]} \times 100 \quad (1)$$

2.2.2. Antimicrobial Assay

The antimicrobial efficacy of each candidate compound was assessed through a precise broth microdilution method. Additionally, the experiment extended to measure the minimum microbicidal concentration (MMC), identifying a minor concentration required to eradicate the microbial population. This dual assessment provides comprehensive insights into the candidates' growth-suppressing and microbe-killing potential, ensuring a robust evaluation of their antimicrobial capabilities. Resazurin served as the indicator in this assay .The test microorganisms included *Escherichia coli* ATCC 25922 [10], *Staphylococcus aureus* ATCC 25923 [11], *Pseudomonas aeruginosa* [12], *Enterococcus faecalis* [13], *Acinetobacter baumannii* [14], *Klebsiella pneumonia* [15], and *Candida albicans* [16]. Clinical isolates of *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Candida albicans* were obtained from patients treated at Maharaj Nakhon Si Thammarat Hospital. For optimal growth, bacterial isolates were cultivated on Trypticase Soy Agar (TSA) at 37°C, whereas *C. albicans* was grown on Sabouraud Dextrose Agar (SDA) at 25°C to suit its unique fungal growth requirements.

All cultures were incubated for 3 hours under these conditions to ensure active growth phases before experimentation. The suspensions were then standardized by diluting them in 0.85% sodium chloride solution until their turbidity matched the 0.5 McFarland standard, establishing uniform microbial density for subsequent analyses. For bacteria, this suspension was further diluted 100-fold before use .Test samples were prepared as DMSO and 2X MHB solutions, with an initial concentration of 2,000 µg/mL. Serial dilutions were performed to achieve lower concentrations, ensuring the DMSO content remained below 10% in each dilution .Microbial suspensions (10 µL) were added to each sample concentration, mixed thoroughly, and incubated at the appropriate temperature for 24 hours. After incubation, 10 µL of resazurin solution was added to each well and incubated for 3 hours. A color change from deep blue to pink indicated .the presence of viable microorganismsThe lowest sample concentration preventing this color change was recorded as the MIC. Aliquots from wells where resazurin color did not change were plated on Mueller Hinton Agar (MHA) for bacteria or SDA for *C. albicans* to determine the MMC. Plates were incubated under the same conditions for 3 hours. Tetracycline HCl and ketoconazole were used as positive controls for bacteria and fungi, respectively [17].

2.2.3. Cytotoxic Activity Assay

The cytotoxic potential of the crude extracts was systematically evaluated using the MTT assay, a well-established method for assessing cell viability and metabolic activity. High-quality reagents, including MTT and DMSO from Sigma-Aldrich and cell culture materials such as DMEM, MEM, and FBS from GIBCO Life Technologies, ensured the reliability of experimental results. The study employed three cell lines: KB (human oral carcinoma), HeLa (human cervical adenocarcinoma), and Vero (African green monkey kidney cells), with the latter serving as a model for non-cancerous cytotoxicity assessment. Cell cultures were mentioned in the previous report. Upon reaching 80% confluency, they were seeded at 3×10^3 cells per well in 96-well plates and allowed to adhere overnight. Treatments involved a range of extract concentrations (1–1,000 µg/mL), and 0.5% DMSO was included as a negative control. After 72 hours of exposure, the metabolic activity of the cells was assessed by adding MTT, which is reduced by mitochondrial enzymes in viable cells to insoluble formazan crystals. The formed crystals were dissolved in DMSO to quantify cell viability, and the absorbance at 550 nm was measured using a microplate reader. This absorbance was directly proportional to the number of metabolically active cells. Percent viability was calculated relative to the control, and IC_{50} values were derived using SigmaPlot software, indicating the concentration required to inhibit 50% of cell viability. The method allowed for a detailed comparison of the cytotoxic effects of the extracts on cancerous and non-cancerous cells, providing valuable insights into their selective toxicity and therapeutic potential [18-23].

3. Results and Discussion

3.1. Extraction

Seventeen medicinal plant species were meticulously selected from mangrove ecosystems in Nakhon Si Thammarat, Thailand, with careful attention to geographical and ecological diversitym (Figure 1). These plants were collected from multiple districts, including Khanom (samples 7–9, 11–14, 17–18, 22–23), Thasala (samples 4–5), Muang District (sample 3), and Pakphanang (samples 1–2, 6, 10, 15–16, 19–21). Twenty-three samples were obtained, representing various plant parts such as leaves, roots, fruit peels, seeds, and stems. This systematic separation allowed a targeted exploration of bioactive compounds specific to each plant part. To ensure accuracy in documentation and facilitate future research, voucher specimens were authenticated and preserved at the Walailak Botanical Park, Walailak University. Each sample underwent a rigorous preparation process, beginning with a thorough cleaning to remove debris. The plant materials were then categorized by type and dried at a consistent temperature of 50 °C using a hot-air oven to preserve their phytochemical integrity. Following drying, the materials were finely ground into powders using a milling device to enhance the efficiency of subsequent extraction. Methanolic extraction was performed using a reflux method, where each powdered sample was treated with methanol in three successive cycles (1.0 L each for 3 hours). This approach maximized the recovery of bioactive compounds. The pooled methanolic extracts were then filtered to remove insoluble materials, and the filtrates were concentrated under reduced pressure to eliminate all volatile components, yielding crude methanolic extracts. The efficiency of the extraction process was quantified, and the percentage yield of each sample is detailed in Table 1, providing insights into the variability in extractable bioactive content across different species and plant parts. This methodical approach not only ensured the reproducibility of the study but also set a strong foundation for subsequent pharmacological analyses. Figure 2 shows the flowchart of the research methodology through which the objectives of this study were achieved.

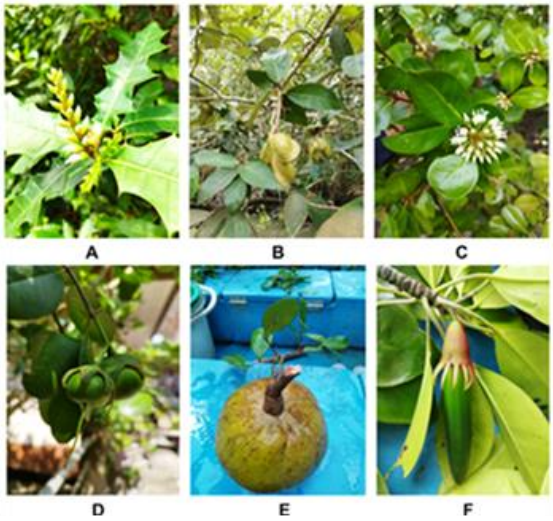

Figure 1. Some medicinal plants from the mangrove forest of Nakhon Si Thammarat, Thailand (**A**: ***Acanthus ebracteatus*; leaves and flowers, B**: ***Heritiera littoralis*; Leaves, stem, and fruits, C**: ***Aegiceras corniculatum*; leaves and flowers, D**: ***Sonneratia griffithii*; Leaves and fruits, E**: ***Xylocarpus granatum*; fruit, and F**: ***Bruguiera gymnorrhiza*; Leaves and fruit).**

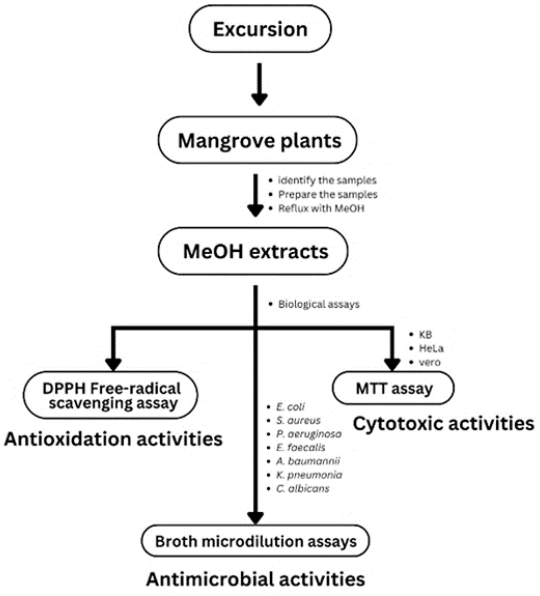

Figure 2. The flowchart of investigation in this research work

Table 1. Methanol extracts of some medicinal plants from the mangrove forests in Nakhon Si Thammarat, Thailand (Sample 1 – 23)

Sample	Medicinal plant name	Family	Part of use	Weight of the powder sample (g)	Weight of the dried MeOH extract (g)	% Yield (w/w)
1	*Aegiceras corniculatum*	Primulaceae	Leaves	135.63	23.32	17.19
2	*Aegiceras corniculatum*	Primulaceae	Stem	132.00	15.67	11.87
3	*Nypa fruticans*	Arecaceae	Fruits	166.65	56.40	33.84
4	*Bruguiera malabarica*	Rhizophoraceae	Leaves	180.14	35.81	19.88
5	*Bruguiera malabarica*	Rhizophoraceae	Stem	58.24	13.98	24.01
6	*Sonneratia ovata*	Lythraceae	Leaves and stem	105.26	22.57	21.45
7	*Acanthus volubilis*	Acanthaceae	Leaves and stem	136.63	13.74	10.05
8	*Acanthus ebracteatus*	Acanthaceae	Leaves and stem	72.33	14.22	19.66
9	*Glochidion littorale*	Euphorbiaceae	Leaves	118.00	11.69	9.91
10	*Rhizophora mucronata*	Rhizophoraceae	Leaves	63.43	5.59	8.81
11	*Xylocarpus granatum*	Meliaceae	Seed	46.00	4.53	9.84
12	*Xylocarpus granatum*	Meliaceae	Peel of fruit	56.00	12.28	21.94
13	*Xylocarpus granatum*	Meliaceae	Root	97.97	25.55	26.07
14	*Xylocarpus moluccensis*	Meliaceae	Root	103.77	36.20	34.89
15	*Sonneratia caseolaris*	Lythraceae	Root	256.32	51.61	20.13
16	*Sonneratia caseolaris*	Lythraceae	Leaves and stem	55.38	8.07	14.58
17	*Heritiera littoralis*	Malvaceae	Fruit	110.43	11.28	10.22
18	*Heritiera littoralis*	Malvaceae	Leaves and stem	105.11	25.04	23.82
19	*Sonneratia griffithii*	Lythraceae	Leaves	105.59	23.72	22.46
20	*Lumnitzera racemosa*	Combretaceae	Leaves	184.74	15.19	8.22
21	*Ceriops decandra*	Rhizophoraceae	Leaves and stem	108.75	22.96	21.12
22	*Bruguiera gymnorrhiza*	Rhizophoraceae	Leaves	99.55	24.25	24.36
23	*Cycas rumphii*	Cycadaceae	Leaves	59.42	8.67	14.60

3.2. Phytochemical Screening

A comprehensive phytochemical analysis was conducted on the methanolic leaf extracts to identify key bioactive compounds. This involved employing a series of standardized qualitative assays tailored to detect phytochemical groups such as alkaloids, flavonoids, tannins, terpenoids, and steroids. The findings provide a broad-spectrum profile of the phytochemical diversity within the collected plant samples. The summarized outcomes of this screening are provided in Table 2, offering a clear comparative overview of the phytochemical constituents across different extracts. Additional details are available in Table 3 for in-depth insights into the methods and results, including the specific color changes, precipitates, or reactions observed during testing. This layered approach to data presentation ensures both accessibility for quick reference and thoroughness for those seeking detailed experimental evidence. By systematically cataloging the phytochemical composition of these extracts, this analysis sets the stage for understanding their potential pharmacological applications and guiding future bioactivity studies. The diverse phytochemical landscape underscores the therapeutic potential of mangrove-derived plant species as promising candidates for drug discovery and development.

3.2.1. Alkaloid Detection

Alkaloids within the methanolic extracts were identified using Dragendorff's reagent, a reliable and widely used chemical assay. To initiate the test, a small portion of each extract was subjected to acid hydrolysis by heating with 5 mL of 10% sulfuric acid in a water bath for 10 minutes. This step facilitated the release of free alkaloids from their bound forms, enhancing their detectability. The Dragendorff's reagent reacts explicitly with alkaloid compounds to produce a characteristic orange-red amorphous precipitate, a visual indicator. Positive results were observed in samples 1–9, 11, 14–18, and 21–23, confirming the presence of alkaloids in these extracts. This method provided a qualitative confirmation of the alkaloid presence and highlighted the variability of alkaloid content across different plant samples. The findings underscore the potential pharmacological significance of these extracts, as alkaloids are well-known for their diverse bioactivities, including antimicrobial, anti-inflammatory, and anticancer properties. Using Dragendorff's reagent in this context offers a straightforward yet practical approach for the preliminary screening of bioactive alkaloid compounds.

3.2.2. Steroid and Terpenoid Detection

The Liebermann–Burchard test, adapted to enhance specificity and reliability, was employed to identify the presence of steroids and terpenoids in the methanolic extracts. The samples were pre-treated with chloroform to eliminate

interference from chlorophylls and other pigments, ensuring a transparent reaction environment. This preparatory step optimized the detection of the targeted compounds by removing extraneous substances that could mask the test results. Upon analysis, all 23 samples demonstrated the presence of terpenoids, a diverse class of bioactive compounds known for their significant pharmacological properties, including anti-inflammatory, antimicrobial, and anticancer activities. Furthermore, the test identified steroids in a subset of samples 2, 13, 14, 16, 19, 20, and 21. Steroids are critical compounds with various biological roles, from hormone regulation to membrane stabilization. This dual identification underscores the chemical diversity within the plant samples and highlights the potential of these extracts as sources of bioactive molecules. The presence of terpenoids and steroids across different samples suggests their relevance in traditional medicine practices and their promise for future pharmaceutical applications. By combining a targeted extraction approach with the modified Liebermann–Burchard test, this study provided a robust framework for exploring the phytochemical richness of natural products.

3.2.3. Tannin Detection

The presence of tannins in methanolic extracts was evaluated using a combination of chemical assays to distinguish between condensed and hydrolysable tannins. Initially, each sample was boiled with 5 mL of distilled water to extract tannins into the aqueous phase. After filtration, the filtrates underwent sequential testing with ammonium hydroxide TS, 1% ferric chloride, and saturated lead subacetate solutions to confirm the presence and type of tannins. This result underscores the prevalence of condensed tannins across the plant species studied compounds often associated with antioxidant and antimicrobial activities. Further analysis using the ferric chloride test identified hydrolysable tannins in a subset of samples: 2, 6, 15, 16, 19, and 20. The development of a dark blue to turquoise-black coloration in these samples provided explicit visual confirmation of their presence. This distinction between tannin types is particularly significant, as hydrolysable tannins possess unique bioactivities, including enzyme inhibition and metal chelation, which differ from the more commonly studied condensed tannins. This comprehensive approach to tannin testing highlights the extracts' chemical diversity and underscores these plant-derived compounds' potential for pharmacological applications. The dual occurrence of tannin types in several samples indicates their synergistic bioactive potential, meriting further investigation into their therapeutic roles.

3.2.4. Flavonoid Detection

The color changes observed during the tannin tests also confirmed the presence of flavonoids in all samples. These findings collectively highlight the diverse phytochemical constituents within the methanolic extracts.

Table 2. Phytochemical screening of the methanolic extracts of some medicinal plants from the mangrove forest in Nakhon Si Thammarat, Thailand (Sample 1 – 23)

Sample	Medicinal plant name	Part of use	Tannins	Flavonoids	Alkaloids	Terpenoids	Steroids
1	*Aegiceras corniculatum*	Leaves	●	●	●	●	
2	*Aegiceras corniculatum*	Stem	●	●	●	●	●
3	*Nypa fruticans*	Fruits	●	●	●	●	
4	*Bruguiera malabarica*	Leaves	●	●	●	●	
5	*Bruguiera malabarica*	Stem	●	●	●	●	
6	*Sonneratia ovata*	Leaves and stem	●	●	●	●	
7	*Acanthus volubilis*	Leaves and stem	●	●	●	●	
8	*Acanthus ebracteatus*	Leaves and stem	●	●	●	●	
9	*Glochidion littorale*	Leaves	●	●	●	●	
10	*Rhizophora mucronata*	Leaves	●	●		●	
11	*Xylocarpus granatum*	Seed	●	●	●	●	
12	*Xylocarpus granatum*	Peel of fruit	●	●		●	
13	*Xylocarpus granatum*	Root	●	●		●	●
14	*Xylocarpus moluccensis*	Root	●	●	●	●	●
15	*Sonneratia caseolaris*	Root	●	●	●	●	
16	*Sonneratia caseolaris*	Leaves and stem	●	●	●	●	●
17	*Heritiera littoralis*	Fruit	●	●	●	●	
18	*Heritiera littoralis*	Leaves and stem	●	●	●	●	
19	*Sonneratia griffithii*	Leaves	●	●		●	●
20	*Lumnitzera racemosa*	Leaves	●	●		●	●
21	*Ceriops decandra*	Leaves and stem	●	●	●	●	●
22	*Bruguiera gymnorrhiza*	Leaves	●	●	●	●	
23	*Cycas rumphii*	Leaves	●	●	●	●	

Table 3. Phytochemical screening of the methanolic extracts of some medicinal plants from the mangrove forest in Nakhon Si Thammarat, Thailand (Sample 1 – 23) (in-depth insights)

Sample	Medicinal plant name	Part of use	1% $FeCl_3$	Sat. lead subacetate	Liebermann–Burchard test	Dragendorff's reagent	Ammonium hydroxide TS
1	*Aegiceras corniculatum*	Leaves	Turquoise precipitate	Precipitate	Magenta	+	Pale yellow solution with yellow precipitate
2	*Aegiceras corniculatum*	Stem	Black precipitate	Precipitate	Blue-green and magenta	++	Pale red-orange solution with yellow precipitate
3	*Nypa fruticans*	Fruits	Turquoise precipitate	Precipitate	Magenta	+	Pale red-orange solution with yellow precipitate
4	*Bruguiera malabarica*	Leaves	Turquoise precipitate	Precipitate	Magenta	+	Pale yellow solution with yellow precipitate
5	*Bruguiera malabarica*	Stem	Turquoise precipitate	Precipitate	Magenta	+	Pale yellow solution with yellow precipitate
6	*Sonneratia ovata*	Leaves and stem	Dark blue precipitate	Precipitate	Magenta	+	Pale yellow solution with yellow precipitate
7	*Acanthus volubilis*	Leaves and stem	Turquoise precipitate	Precipitate	Magenta	+	Pale yellow solution with yellow precipitate
8	*Acanthus ebracteatus*	Leaves and stem	Turquoise precipitate	Precipitate	Magenta	+	Pale yellow solution with yellow precipitate
9	*Glochidion littorale*	Leaves	Turquoise precipitate	Precipitate	Magenta	+	Pale yellow solution with yellow precipitate
10	*Rhizophora mucronata*	Leaves	Turquoise precipitate	Precipitate	Magenta	-	Pale red-orange solution with yellow precipitate
11	*Xylocarpus granatum*	Seed	Turquoise precipitate	Precipitate	Magenta	++	Pale red-orange solution with yellow precipitate
12	*Xylocarpus granatum*	Peel of fruit	Turquoise precipitate	Precipitate	Magenta	-	Red-brown solution
13	*Xylocarpus granatum*	Root	Turquoise precipitate	Precipitate	Blue-green and magenta	-	Red-brown solution
14	*Xylocarpus moluccensis*	Root	Turquoise precipitate	Precipitate	Blue-green and magenta	+	Pale red-orange solution with yellow precipitate
15	*Sonneratia caseolaris*	Root	Black precipitate	Precipitate	Magenta	+	Pale yellow solution with yellow precipitate
16	*Sonneratia caseolaris*	Leaves and stem	Dark blue precipitate	Precipitate	Blue-green and magenta	++	Pale yellow solution with white precipitate
17	*Heritiera littoralis*	Fruit	Turquoise precipitate	Precipitate	Magenta	++	Pale yellow solution with white precipitate
18	*Heritiera littoralis*	Leaves and stem	Turquoise precipitate	Precipitate	Magenta	+	Pale red-orange solution with yellow precipitate
19	*Sonneratia griffithii*	Leaves	Black precipitate	Precipitate	Blue-green and magenta	-	Pale yellow solution with yellow precipitate
20	*Lumnitzera racemosa*	Leaves	Black precipitate	Precipitate	Blue-green and magenta	-	Pale red-orange solution with yellow precipitate
21	*Ceriops decandra*	Leaves and stem	Turquoise precipitate	Precipitate	Blue-green and magenta	+	Pale red-orange solution with yellow precipitate
22	*Bruguiera gymnorrhiza*	Leaves	Turquoise precipitate	Precipitate	Magenta	++	Pale yellow solution with white precipitate
23	*Cycas rumphii*	Leaves	Turquoise precipitate	Precipitate	Magenta	+	Pale yellow solution with yellow precipitate

Remarks: -: negative, +: positive, and ++: amount estimated visually.

3.3. Biological Evaluation

3.3.1. Antioxidant Activity

The antioxidant potential of the methanolic extracts (Samples 1–23) was evaluated using the DPPH-based free-radical-scavenging assay. The antioxidant activity of the methanolic extracts was compared to L-ascorbic acid, which served as the positive control. The results indicate that several extracts demonstrated significant antioxidant activity, with $\%SC_{50}$ values surpassing L-ascorbic acid. Notably, Sample 6 ($\%SC_{50} = 5.00 \pm 0.08$ µg/mL), Sample 19 ($\%SC_{50} = 5.15 \pm 0.15$ µg/mL), Sample 13 ($\%SC_{50} = 5.87 \pm 0.06$ µg/mL), Sample 21 ($\%SC_{50} = 6.14 \pm 0.06$ µg/mL), and Sample 14 ($\%SC_{50} = 6.28 \pm 0.93$ µg/mL) exhibited the highest activity among the tested samples. The complete results of the DPPH-based free-radical-scavenging assay for all samples are provided in Table 4.

Table 4. Antioxidant activity of the methanol crude extracts of some medicinal plants from the mangrove forest in Nakhon Si Thammarat, Thailand (Sample 1 – 23) and L-ascorbic acid

Sample	Medicinal plant name	Part of use	% SC_{50} (µg/mL)
1	*Aegiceras corniculatum*	Leaves	20.82 ± 0.39
2	*Aegiceras corniculatum*	Stem	26.38 ± 0.14
3	*Nypa fruticans*	Fruits	59.14 ± 0.35
4	*Bruguiera malabarica*	Leaves	189.89 ± 2.11
5	*Bruguiera malabarica*	Stem	79.35 ± 2.93
6	*Sonneratia ovata*	Leaves and stem	5.00 ± 0.08
7	*Acanthus volubilis*	Leaves and stem	137.37 ± 1.00
8	*Acanthus ebracteatus*	Leaves and stem	65.27 ± 0.50
9	*Glochidion littorale*	Leaves	47.88 ± 0.88
10	*Rhizophora mucronata*	Leaves	33.94 ± 4.37
11	*Xylocarpus granatum*	Seed	18.29 ± 0.19
12	*Xylocarpus granatum*	Peel of fruit	7.61 ± 0.05
13	*Xylocarpus granatum*	Root	5.87 ± 0.06
14	*Xylocarpus moluccensis*	Root	6.28 ± 0.93
15	*Sonneratia caseolaris*	Root	10.49 ± 0.66
16	*Sonneratia caseolaris*	Leaves and stem	9.28 ± 0.06
17	*Heritiera littoralis*	Fruit	61.05 ± 0.93
18	*Heritiera littoralis*	Leaves and stem	10.03 ± 0.20
19	*Sonneratia griffithii*	Leaves	5.15 ± 0.15
20	*Lumnitzera racemosa*	Leaves	12.03 ± 0.11
21	*Ceriops decandra*	Leaves and stem	6.14 ± 0.06
22	*Bruguiera gymnorrhiza*	Leaves	27.95 ± 0.34
23	*Cycas rumphii*	Leaves	94.45 ± 3.06
24	L-ascorbic acid		6.78 ± 0.07

3.3.2. Antimicrobial Activity

Resazurin served as the indicator for microbial viability. Tetracycline HCl and ketoconazole were positive controls for antibacterial and antifungal activity. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extracts that inhibited microbial growth. In contrast, the minimum microbicidal concentration (MMC) was the lowest, resulting in microbial death. A summary of the antimicrobial activity results, including MIC and MMC values, is presented in Table 5.

Several methanolic extracts demonstrated bacteriostatic activity against the tested microbes. Among these, Sample 19 exhibited the most substantial bacteriostatic effect against *Staphylococcus aureus* with a MIC of 62.50 µg/mL, followed by Samples 6, 12, and 13, each with a MIC of 125.00 µg/mL. Additionally, Samples 2 (MIC = 250.00 µg/mL), Sample 21 (MIC = 500.00 µg/mL), and Samples 1, 11, and 18 (MIC = 1,000.00 µg/mL) also showed bacteriostatic activity against *S. aureus*. For *Acinetobacter baumannii*, Samples 2, 6, 16, and 19 showed the highest bacteriostatic activity, each with a MIC of 62.50 µg/mL. These extracts exhibited the most potent activity against this microbe among all tested samples. In the case of *Pseudomonas aeruginosa*, a common cause of hospital-acquired infections, several methanolic extracts demonstrated bacteriostatic effects. Samples 6 and 19 showed the highest activity with a MIC of 62.50 µg/mL. Other samples, including 16 and 20 (MIC = 125.00 µg/mL), Sample 15 (MIC = 250.00 µg/mL), and Sample 2 (MIC = 500.00 µg/mL), also inhibited *P. aeruginosa*. Against *Enterococcus faecalis*, the methanolic extract of Sample 2 was the most effective, with a MIC of 250.00 µg/mL. Samples 16 and 19 also inhibited the growth of *E. faecalis*, each with a MIC of 500.00 µg/mL. Unfortunately, the methanolic extracts from all tested samples were ineffective in inhibiting or killing *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans*.

Table 5. Results of a broth microdilution assay of some mangrove plants (Sample 1 – 23) and the positive controls

Sample	Medicinal plant name	Part of use	MIC/MMC; µg/mL						
			S. aureus	*E. coli*	*A. baumannii*	*K. pneumoniae*	*P. aeruginosa*	*E. faecalis*	*C.albicans*
1	*Aegiceras corniculatum*	Leaves	1,000/ND	NO	NO	NO	1,000/ND	NO	NO
2	*Aegiceras corniculatum*	Stem	250/ND	NO	62.50/ND	NO	500/ND	250/ND	NO
3	*Nypa fruticans*	Fruits	NO	NO	NO	NO	NO	NO	NO
4	*Bruguiera malabarica*	Leaves	NO	NO	NO	NO	NO	NO	NO
5	*Bruguiera malabarica*	Stem	NO	NO	NO	NO	NO	NO	NO
6	*Sonneratia ovata*	Leaves and stem	125/ND	NO	62.50/ND	NO	62.50/ND	NO	NO
7	*Acanthus volubilis*	Leaves and stem	NO	NO	NO	NO	NO	NO	NO
8	*Acanthus ebracteatus*	Leaves and stem	NO	NO	NO	NO	1,000/ND	NO	NO
9	*Glochidion littorale*	Leaves	NO	NO	NO	NO	1,000/ND	NO	NO
10	*Rhizophora mucronata*	Leaves	NO	NO	NO	NO	1,000/ND	NO	NO
11	*Xylocarpus granatum*	Seed	1,000/ND	NO	NO	NO	1,000/ND	NO	NO
12	*Xylocarpus granatum*	Peel of fruit	125/ND	NO	NO	NO	1,000/ND	NO	NO
13	*Xylocarpus granatum*	Root	125/ND	NO	NO	NO	1,000/ND	NO	NO
14	*Xylocarpus moluccensis*	Root	NO	NO	NO	NO	1,000/ND	NO	NO
15	*Sonneratia caseolaris*	Root	NO	NO	1,000/ND	NO	250/ND	NO	NO
16	*Sonneratia caseolaris*	Leaves and stem	NO	NO	62.50/ND	NO	125/ND	500/ND	NO
17	*Heritiera littoralis*	Fruit	NO	NO	NO	NO	NO	NO	NO
18	*Heritiera littoralis*	Leaves and stem	1,000/ND	NO	NO	NO	1,000/ND	NO	NO
19	*Sonneratia griffithii*	Leaves	62.50/ND	NO	62.50/ND	NO	62.50/ND	500/ND	NO
20	*Lumnitzera racemosa*	Leaves	NO	NO	NO	NO	125/ND	NO	NO
21	*Ceriops decandra*	Leaves and stem	500/ND	NO	NO	NO	1,000/ND	NO	NO
22	*Bruguiera gymnorrhiza*	Leaves	NO	NO	NO	NO	NO	NO	NO
23	*Cycas rumphii*	Leaves	NO	NO	NO	NO	1,000/ND	NO	NO
25	Tetracycline HCl		0.03/15.63	0.49/15.63	62.50/125	125/125	3.91/15.63	0.24/0.98	-
26	Ketoconazole		-	-	-	-	-	-	31.25/NO

ND = not determined; although very weak inhibition was observed, the values could not be determined in the tested concentration range, due to low activity. NO = not observed; no inhibition was observed at the tested highest concentration.

3.3.3. Cytotoxic Activity Assay

The cytotoxic activities of the methanolic extracts were evaluated using the MTT assay, with IC_{50} values determined for two cancer cell lines (KB and HeLa) and one regular cell line (Vero). The IC_{50} values for each sample are summarized in Table 6.

KB Cells:

The IC_{50} values for KB cells ranged from 8.48 ± 1.91 µg/mL to greater than 1,000 µg/mL. Notably, Samples 2, 4, 6, 7, 8, 18, 19, 20, 21, and 23 exhibited IC_{50} values below 200 µg/mL, with values ranging from 8.48 ± 1.91 µg/mL to 164.77 ± 37.25 µg/mL.

HeLa Cells:

For HeLa cells, the IC_{50} values ranged from 55.04 ± 8.28 µg/mL to greater than 1,000 µg/mL. Samples 1, 2, 3, 4, 8, 9, 10, 13, 15, 17, 18, 19, 22, and 23 demonstrated IC_{50} values below 200 µg/mL, ranging from 55.04 ± 8.28 µg/mL to 181.64 ± 14.16 µg/mL.

Vero Cells (Normal):

The IC_{50} values for the Vero cell line ranged from 25.67 ± 15.56 µg/mL to greater than 1,000 µg/mL. Among the 23 tested samples, 17 exhibited IC_{50} values above 200 µg/mL, indicating lower toxicity to normal cells.

Selectivity Index (SI):

The selectivity index (SI) was calculated as the ratio of the IC_{50} value in Vero cells to the IC_{50} value in cancer cells.

- KB Cell Line: SI values ranged from 0.34 to 56.12. Samples 19, 20, 21, 7, 8, 4, 2, and 18 displayed high selectivity, with SI values between 56.12 and 5.88.
- HeLa Cell Line: SI values ranged from 1 to 16.86. Samples 4, 22, 3, 17, 15, 19, 9, 2, 16, 1, 5, 11, and 20 showed high selectivity, with SI values between 16.86 and 3.31.

Four samples (2, 4, 19, and 20) exhibited high selectivity for both KB and HeLa cells. Among these, Sample 19 showed the highest selectivity, with an SI value of 56.12 for KB cells and 6.22 for HeLa cells. This study marks the first report of biological activities for all candidates, with particular emphasis on the potent and selective activity of Sample 19.

Table 6. **Results of a cytotoxic activity assay with MTT assay of the methanol crude extract of some medicinal plants from the mangrove forest in Nakhon Si Thammarat, Thailand (Sample 1 – 23)**

Sample	Medicinal plant name	Part of use	IC_{50} (µg/mL)		
			KB	HeLa	Vero
1	*Aegiceras corniculatum*	Leaves	765.29 ± 182.48	55.04 ± 8.26	264.23 ± 22.38
2	*Aegiceras corniculatum*	Stem	164.77 ± 37.25	174.38 ± 14.56	> 1,000
3	*Nypa fruticans*	Fruits	>1,000	80.20 ± 15.88	> 1,000
4	*Bruguiera malabarica*	Leaves	147.34 ± 41.44	59.31 ± 2.45	>1,000
5	*Bruguiera malabarica*	Stem	>1,000	237.24 ± 28.30	> 1,000
6	*Sonneratia ovata*	Leaves and stem	163.27 ± 10.34	264.23 ± 22.38	126.97 ± 70.78
7	*Acanthus volubilis*	Leaves and stem	45.70 ± 29.23	>1,000	> 1,000
8	*Acanthus ebracteatus*	Leaves and stem	8.48 ± 1.91	181.64 ± 14.160	63.66 ± 17.04
9	*Glochidion littorale*	Leaves	> 1,000	163.23 ± 19.14	> 1,000
10	*Rhizophora mucronata*	Leaves	> 1,000	88.43 ± 14.47	25.67 ± 15.59
11	*Xylocarpus granatum*	Seed	> 1,000	282.03 ± 31.20	> 1,000
12	*Xylocarpus granatum*	Peel of fruit	> 1,000	390.49 ± 45.14	663.78 ± 39.85
13	*Xylocarpus granatum*	Root	> 1,000	90.30 ± 10.89	91.71 ± 20.63
14	*Xylocarpus moluccensis*	Root	> 1,000	266.84 ± 35.19	249.56 ± 48.55
15	*Sonneratia caseolaris*	Root	>1,000	109.65 ± 36.65	> 1,000
16	*Sonneratia caseolaris*	Leaves and stem	>1,000	207.51 ± 11.13	> 1,000
17	*Heritiera littoralis*	Fruit	>1,000	100.32 ± 29.65	> 1,000
18	*Heritiera littoralis*	Leaves and stem	10.51 ± 1.85	130.63 ± 63.66	61.80 ± 3.98
19	*Sonneratia griffithii*	Leaves	17.82 ± 4.91	160.68 ± 39.36	> 1,000
20	*Lumnitzera racemosa*	Leaves	18.54 ± 7.24	302.44 ± 85.11	> 1,000
21	*Ceriops decandra*	Leaves and stem	41.86 + 8.77	>1,000	> 1,000
22	*Bruguiera gymnorrhiza*	Leaves	>1,000	69.89 ± 8.23	> 1,000
23	*Cycas rumphii*	Leaves	108.80 ± 16.18	166.38 ± 39.90	135.87 ± 44.49

Remarks: IC_{50} values are the concentrations that inhibit cell growth 50% of the control. Each value represents the mean of sextuplicate results ± SD.

3.4. The Example of the Curve of %SC and Concentration of the Test Sample

The sample solutions were prepared at five different concentrations, with each concentration tested in triplicate. The reactions with DPPH were performed, and the absorbance values were corrected by subtracting the corresponding sample blanks to eliminate any interference from the samples themselves. The resulting data were then used to construct a curve after 30 minutes of reaction, with absorbance measured at 517 nm. The mean values obtained were plotted to generate the curve shown, from which the regression equation was derived for subsequent calculation of the $\%SC_{50}$.

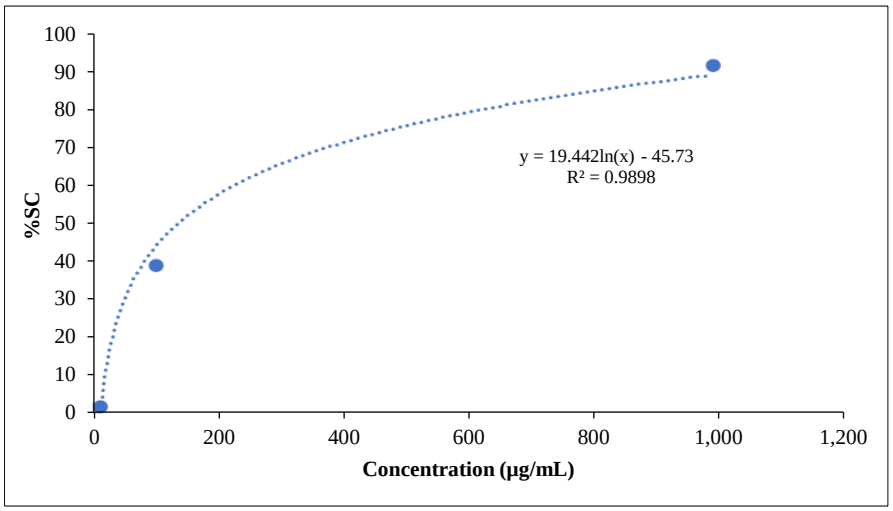

Figure 3. The relationship between %SC and concentration of Sample 6

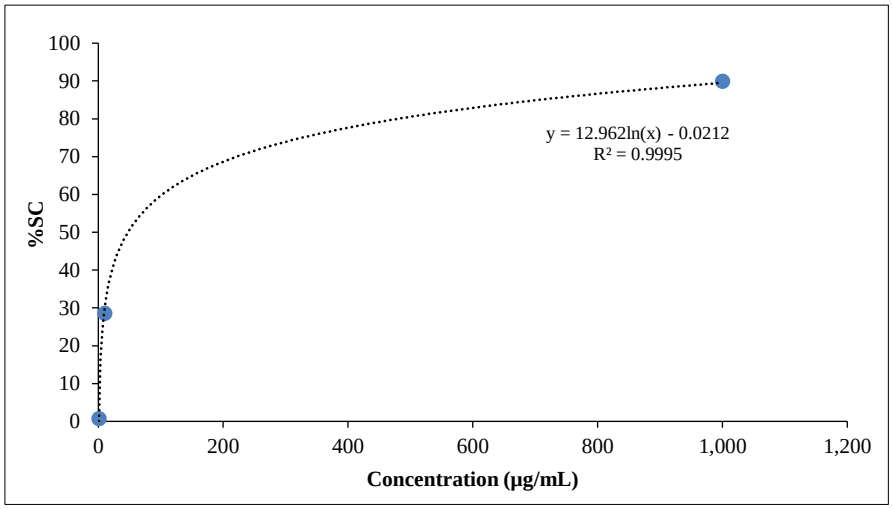

Figure 4. The relationship between %SC and concentration of Sample 7

3.5. Cytotoxic Activities Assay

The IC_{50} or 50 %inhibitory concentration was calculated by using Sigmaplot 10.0 Software .The mean and standard deviation of IC_{50} were calculated from six graphs (Figure 5).

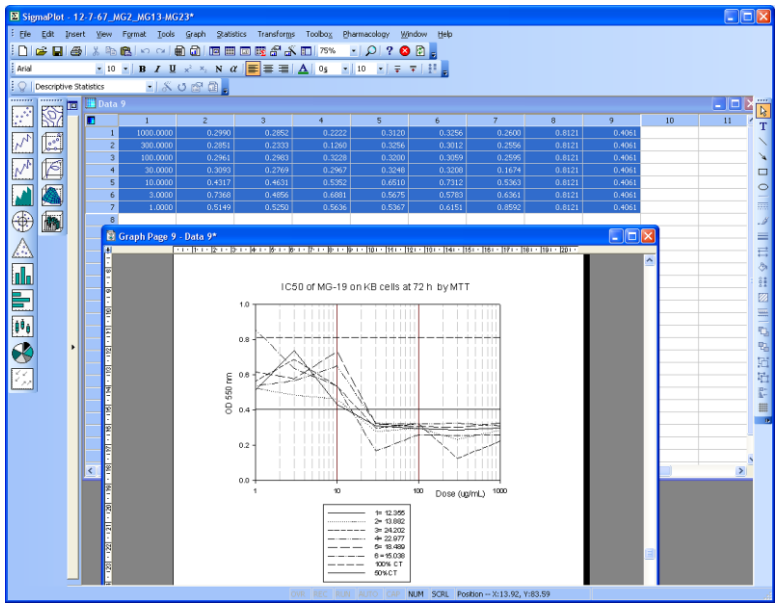

Figure 5. The captured photo of the Sigmaplot 10.0 Software during the process of the data

4. Conclusion

Heat-assisted extraction, such as refluxing with solvents, yielded crude extracts containing nearly all sample constituents. The methanolic extract yields ranged between 8.22% and 34.89%, depending on the type and part of the plant used. All samples exhibited the presence of tannins. Specifically, the reaction with 1% $FeCl_3$ resulted in a turquoise color, indicating the presence of condensed tannins, while a blue color signified hydrolyzable tannins. Additionally, the reaction with ammonium hydroxide TS confirmed the presence of flavonoids in all samples. The appearance of an orange or orange-red color after reacting with Dragendorff's reagent indicated alkaloids found in samples 1–9, 11, 14–18, and 21–23.

The Liebermann–Burchard test revealed that all samples contained terpenoids, while steroids were identified in samples 2, 13, 14, 16, and 19–21. These findings supported the antioxidant activities of the samples, as functional groups in flavonoids and hydrolyzable tannins contain lone-pair electrons that neutralize free radicals. Samples 6, 19, 13, 21, and 14 exhibited the highest antioxidant activities, surpassing the activity of L-ascorbic acid.

The results also highlighted the influence of plant parts on antioxidant activity. For instance, in *Bruguiera malabarica*, the stem showed approximately three times stronger activity than the leaves. Similarly, in *Heritiera littoralis*, the leaves and stems demonstrated better antioxidant activity than the fruits. Specifically, Sample 18 (*Heritiera littoralis*: leaves and stem) had a $\%SC_{50}$ value of 10.03 ± 0.20 µg/mL, compared to Sample 17 (*Heritiera littoralis*: fruits), which had a $\%SC_{50}$ value of 61.05 ± 0.93 µg/mL, a difference of approximately sixfold.

In antimicrobial assays, Sample 19 showed the most significant activity against *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* with a MIC of 62.50 µg/mL. Methanolic extracts from the same genus as Sample 19 also demonstrated promising antimicrobial and antioxidant potential, making them strong candidates for further investigation.

Cytotoxicity and safety profiles were evaluated using the MTT assay on three cell lines: KB (human oral carcinoma), HeLa (human cervical adenocarcinoma), and Vero (normal African green monkey kidney cells). The selectivity of the extracts was confirmed by calculating the IC_{50} ratio (Vero/KB or Vero/HeLa). Ideal candidates were defined as having an IC_{50} against Vero above 1,000 µg/mL and a ratio greater than 3. Based on these criteria, Samples 2, 4, 19, and 20 were identified as potential candidates for selective and safe anticancer activity.

This study emphasizes the importance of exploring natural products' polypharmacophoric properties, particularly antioxidants, antimicrobial agents, and anticancer activities. Microbial infections associated with nitric oxide and oxygen-free radicals often lead to degenerative diseases. Compounds with antimicrobial and antioxidant activities could serve as promising candidates for synergistic therapies.

Among the 17 species and 23 samples of mangrove plants from Nakhon Si Thammarat, Sample 19 emerged as the most promising candidate, showcasing vigorous biological activity. This is the first report on the biological activities of Sample 19, and the findings of this research serve as a valuable guide for drug discovery efforts focused on mangrove plants.

5. Declarations

5.1. Author Contributions

Conceptualization, B.W., S.P., J.N., and K.K.; methodology, B.W., S.P., J.N., and K.K.; validation, B.W.; formal analysis, B.W.; data curation, B.W.; writing—original draft preparation, B.W.; writing—review and editing, B.W., S.P., J.N., and K.K.; visualization, B.W., S.P., J.N., and K.K. All authors have read and agreed to the published version of the manuscript.

5.2. Data Availability Statement

The data presented in this study are available in the article.

5.3. Funding

This research was funded by the Plant Genetic Conservation Project under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG), RSPG-WU-7/2566.

5.4. Acknowledgments

The authors would like to express their gratitude to Mr. Sangiam Tamrariang who is the director of the Mangrove Management Center (Nakhon Si Thammarat), Department of Marine and Coastal Resources, Ministry of Natural Resources and Environment, Thailand, for his support during the field excursions and for providing samples for this study. Special thanks are also extended to Mr. Amorn Makchoo, Mr. Thoedsak Hnuplot, and Mr. Anan Thongthaphae for their assistance during fieldwork and for providing additional samples. The authors also acknowledge the Center for Scientific Equipment and Technology at Walailak University for providing access to essential equipment and facilities.

5.5. Institutional Review Board Statement

Not applicable.

5.6. Informed Consent Statement

Not applicable.

5.7. Declaration of Competing Interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript. Furthermore, all ethical considerations, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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