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Antibacterial activity of selected medicinal plants from Malaysia

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Abstract

Natural sources such as medicinal plants are potential sources of new antibacterial agents to overcome drug resistance and adverse effects. This study aims to investigate the fruit of *Abelmoschus esculentus* (*Malvaceae*; okra), the aerial parts of *Basella alba* (*Basellaceae*; Malabar spinach) and *Nasturtium officinale* (*Brassicaceae*; watercress), the leaves of *Citrus microcarpa* (*Rutaceae*; calamansi) and *Clerodendrum calamitosum* (*Lamiaceae*; white butterfly bush), and the seeds and pods of *Parkia speciosa* (*Leguminosae*; stink bean) for antibacterial activity. To obtain a wide polarity range of secondary metabolites, the plant materials were extracted sequentially using six solvents of increasing polarity. Each plant extract was evaluated against five species of human pathogenic bacteria. The results showed that all six medicinal plants possessed antibacterial activity but with different degrees of bacteriostatic and bactericidal activities. Among the 42 extracts, 76.2% of them showed bacteriostatic activity while only 20 extracts (47.6%) exhibited bactericidal activity. The strongest bacteriostatic activity was shown by the hexane and chloroform extracts of *N. officinale* against *Pseudomonas aeruginosa* with a minimum inhibitory concentration value of 0.02 mg/mL. The highest total activity was given by the hexane extract of *C. microcarpa* against *Bacillus cereus* (56.84 mL/g). For *P. speciosa*, all six extracts from the pods showed bacteriostatic activity while only the hexane and ethanol extracts from the seeds were active against the bacteria. The extracts of *B. alba* and *N. officinale* warrant further isolation and identification of bioactive compounds as they exhibited the broadest bactericidal activity (minimum bactericidal concentration: 0.02-2.50 mg/mL) against all bacterial species.

Keywords: Minimum inhibitory concentration, Minimum bactericidal concentration, *Abelmoschus esculentus*, *Basella alba*, *Citrus microcarpa*, *Clerodendrum calamitosum*, *Nasturtium officinale*, *Parkia speciosa*

1. Introduction

Bacterial resistance to current antimicrobials is a concern to public health as it has detrimental impact on chemotherapy. Some bacteria have developed genetically transmissible defence mechanisms against antimicrobials that cause the antimicrobials to be ineffective against these bacteria. The instances of antimicrobial-resistant bacteria that has been isolated has been increasing globally with isolates that have displayed increased resistance to antimicrobial therapy. [1]. Literature has also indicated the clinical efficacy of some antimicrobials have been negated due to the evolution of these resistant strains. This problem is evident in the bacterial infections such as urinary tract infections, skin and bone infections, pneumonia, bacteremia, endocarditis, and hospital-acquired infections caused by *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species, collectively known as ESKAPE pathogens [2].

Besides that, current antimicrobials are also often associated with detrimental side effects and sometimes adverse effects to the host, for example, anaphylaxis has been associated with the use of penicillins, pulmonary toxicity with nitrofurantoin, arrhythmias with macrolides, and tendonitis, abdominal aorta rupture, and peripheral neuropathy with fluoroquinolones [3]. The search for new antibacterial agents is thereby an ongoing effort, either by design and synthesis of new chemical agents or through the search for natural sources for as-yet

undiscovered bioactive compounds. Plants have long been a valuable natural biomedical source for for the maintenance of health. According to the World Health Organization [4], more than three-quarters of the world population use medicinal plants for some aspects in primary health care. The use of plant extracts and phytochemicals have contributed to the efficacy of treatments [5]. Many plants have been used because of their antimicrobial traits: which are due to compounds synthesized in the secondary metabolism of the plants. These secondary metabolites, such as alkaloids, flavonoids, tannins, and phenolic acids have been shown to possess antibacterial activity [5]. Hence, studies about the use of plants for medicinal purposes should be emphasized, especially those related to the control of drug-resistant bacteria. The following paragraphs will introduce the various plants that form the research focus for this study.

Abelmoschus esculentus (L.) Moench is an annual shrub belonging to the family *Malvaceae*. It is commonly known as okra, lady's finger, or *bendi* (the Malay term). The plant is cultivated for its edible fruit in tropical and subtropical regions [6]. The fruit is 15-20 cm long, pyramidal-oblong in shape with five angles in cross-section, and is hispid. Traditionally, the fruit is used to treat chronic dysentery, gonorrhea, urinary discharges, strangury, and diarrhea [7].

Basella alba L. (*Basellaceae*) is a perennial twining leafy vegetable commonly known as Indian spinach, Malabar spinach, Ceylon spinach, or vine spinach [8]. It is native to tropical Southern Asia and is cultivated widely in Malaysia, the Philippines, tropical Africa, the Caribbean, and tropical South America [9]. The stem is fleshy, and is stout at the base with slender upper branches, and the leaves are axillary, dark green, acute, and is broadly ovate in shape. The fresh leaves and stems are edible and reported to contain protein, vitamins, essential minerals, amino acids, and flavones [10]. Various parts of the plant have been used traditionally for varied medicinal purposes by the people of India, Bangladesh, Nepal, China, Thailand, Malaysia, Nigeria, Kenya, and Cameroon [8,9].

Citrus microcarpa Thunb. (calamansi or calamondin) is a small-size citrus from the family *Rutaceae*. It is widely grown in India, southern Asia, and Southeast Asia especially in Malaysia and Vietnam. Locally known as *limau kasturi*, this fruit is usually harvested to be made into juice and beverages due to its high vitamin C content [11]. The leaves are evergreen, alternate, aromatic, broad-oval, dark-green, glossy on the upper surface, yellowish-green beneath, and 4-7.5 cm long. A decoction of the leaves has been used to treat hypertension and diabetes [12].

Clerodendrum calamitosum L. (*Lamiaceae*), commonly called white butterfly bush, is a small upright woody flowering shrub native to Indonesia. The leaves are simple and opposite arrangement, elliptical to oblong and serrated along margins. The leaves and stem have been used in Taiwanese traditional medicine to treat bladder, kidney, and gall stone problems and as a diuretic [13].

Nasturtium officinale R.Br. (*Brassicaceae*) is a perennial, aquatic or semi-aquatic herb commonly known as watercress. It has hollow, irregular, creeping, or floating stems. The leaves are alternate, compound with three to nine leaflets. Leaves and young shoots can be eaten raw or cooked, as it is rich in vitamins [14]. In Malaysia, the decoction prepared from the leaves and stems is used as an antiscorbutic, diuretic, laxative, and as a remedy for mouth ulcers, cough, and hypertension [15].

Parkia speciosa Hassk. (*Leguminosae*) is a rainforest tree that grows to a height of up to 40 m and is commonly found in India, Malaysia, Indonesia, Thailand, Singapore, the Philippines, Madagascar, and Africa [16]. The fruit comprises a flat, oblong pod with 15-18 bright green, plump, elliptical, or round seeds. The seeds are edible and have a strong and pungent odor; thus, the plant is called stink bean or bitter bean. The seeds can be eaten raw, cooked, or roasted [17]. The fruit is used by the local aborigines for treating diabetes [18].

In this study, the six medicinal plants were selected for the evaluation of antibacterial activity. The plant materials were subjected to sequential solvent extraction using six solvents of increasing polarity. The plant secondary metabolites are segregated into different extractants based on their polarity and solubility during sequential solvent extraction. Less polar solvents such as hexane and chloroform could extract alkaloids, coumarins, fatty acids, and terpenoids while more polar solvents such as ethyl acetate, ethanol, methanol, and water could yield saponins, tannins, flavones, polyphenols, terpenoids, anthocyanins, polypeptides, and lectins from plants [5]. The objectives of this study were to determine the bacteriostatic and bactericidal activities and to calculate the total activity of the plant extracts against a panel of Gram-positive and Gram-negative bacteria. The activities were evaluated using minimum inhibitory concentration and minimum bactericidal concentration assays, respectively.

2. Materials and methods

2.1 Chemicals and reagents

The chemicals and reagents used in the study were as follows: chloramphenicol (Amresco, Ohio, USA); iodonitrotetrazolium chloride (Sigma Aldrich, St. Louis, USA); Mueller-Hinton agar and Mueller-Hinton broth (Oxoid, Hampshire, UK); ethanol 95% (Kofa Chemical Works, Kuala Lumpur, Malaysia); acetone, chloroform,

and ethyl acetate (AR grade, Merck, Darmstadt, Germany); hexane and methanol (AR grade, Mallinckrodt Chemicals, Chesterfield, UK).

2.2 Plant materials

The fruits of *Abelmoschus esculentus* and *Parkia speciosa* were obtained from the markets in Kepong, Kuala Lumpur, and Tapah, Perak, respectively. The aerial parts of *Basella alba* and *Nasturtium officinale* were sourced from wet markets in Ipoh, Perak. The leaves of *Citrus microcarpa* and *Clerodendrum calamitosum* were harvested from the residents staying in Kepong Baru, Kuala Lumpur, and Sungai Ara, Penang, respectively. The species identity of the plants was ascertained by Professor Hean Chooi Ong, an ethnobotanist formerly affiliated with the University of Malaya, Malaysia. Specimen vouchers of the plants were prepared and deposited at the Faculty of Science, Universiti Tunku Abdul Rahman, Malaysia. The specimen code was UTAR/FSC/08/006 for *A. esculentus*, UTAR/FSC/08/012 for *B. alba*, UTAR/FSC/08/005 for *C. microcarpa*, UTAR/FSC/08/009 for *C. calamitosum*, UTAR/FSC/08/003 for *N. officinale*, and UTAR/FSC/08/004 for *P. speciosa*.

2.3 Preparation of plant extracts

The plant samples (326-641 g) were washed under running tap water to remove dirt and dust. The samples were cut into smaller pieces and blended prior to solvent extraction. The solvents used were hexane, chloroform, ethyl acetate, ethanol, methanol, and distilled water, in a sequence of increasing solvent polarity. The maceration (1 L of each solvent) was performed at room temperature for 48 h in the first round and 24 h for the second round. The solvent filtrates were collected from each round and concentrated using a rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland) at 40 °C. The concentrated extracts were then dried in a vacuum concentrator (Concentrator Plus, Eppendorf AG, Hamburg, Germany). The percentage of yield of each extract is calculated as (dry weight of extract/fresh weight of plant sample) x 100. For storage purposes, the dry extracts were kept at -20 °C. For the minimum inhibitory concentration (MIC) assay, each extract was dissolved in a methanol-water mixture (2:1, v/v) to achieve a stock solution of 10 mg/mL. The extract stock solutions were sterilized using 0.45 µm nylon syringe filters before use.

2.4 Bacterial species

Five species of human pathogenic bacteria covering the Gram-positive and Gram-negative groups were selected for the MIC assay. *Bacillus cereus* ATCC 11778™ and *Staphylococcus aureus* ATCC 6538™ were the Gram-positive bacteria used while the Gram-negative bacteria included *Escherichia coli* ATCC 35218™, *Klebsiella pneumoniae* ATCC 13883™, and *Pseudomonas aeruginosa* ATCC 27853™. All bacteria were purchased from the American Type Culture Collection (Manassas, VA, USA). The bacteria were maintained on Mueller-Hinton agar (MHA) plates at 4 °C prior to the assay.

2.5 Preparation of bacterial inoculum

The bacteria were subcultured on MHA and incubated at 37 °C for 24 h before inoculum preparation. Three to five single bacterial colonies were picked from the subcultured plates and mixed with 3 mL of Mueller-Hinton broth (MHB) in a sterile tube. The absorbance of the suspension at the wavelength of 625 nm was adjusted to 0.08-0.10, which corresponds to $1x10^8$ cfu/mL, using a spectrophotometer (Genesys™ 20, Thermo Spectronic, Rochester, NY, USA). The bacterial suspension was further diluted with MHB to achieve the required concentration of $1x10^6$ cfu/mL for the MIC assay.

2.6 Minimum inhibitory concentration (MIC) assay

The MIC of plant extracts was measured using a broth microdilution method [19] with slight modifications. The assay was conducted using sterile 96-well, U-bottom microplates. The plant extract stock solution was two-fold serially diluted with MHB in the microplate to produce eight final concentrations (0.02-2.50 mg/mL) for the assay. Chloramphenicol, which served as a positive control in this study, was diluted similarly to produce a concentration range of 1-128 µg/mL. Several controls were also included in each microplate; MHB alone (100 µL) served as sterility control. Each growth control well contained 50 µL of MHB. Wells for negative control comprised 75 µL of MHB and 25 µL of the respective plant extract stock solutions. After that, 50 µL of the prepared bacterial suspension was inoculated into test sample, positive control, and growth control wells, making the final bacterial suspension to be $5x10^5$ cfu/mL and the final volume of each well to be 100 µL. The microplate was incubated at 37 °C for 24 h. A growth indicator dye called iodonitrotetrazolium (20 µL of 0.4 mg/mL) was introduced to each well of the microplate after incubation. The microplate was further incubated at

37 °C for 30 min to allow color changes to take place. Viable bacterial cells will reduce the yellowish soluble iodonitrotetrazolium to red insoluble iodonitrotetrazolium formazan or precipitate. The lowest concentration of extract with no red precipitate formed in the well is recorded as the MIC value. The MIC assay was carried out in triplicate for each plant extract against each bacterial species.

2.7 Minimum bactericidal concentration (MBC) assay

The spread plate method was used to determine the MBC value of the plant extracts. Twenty microliters of the content from wells designated as MIC or higher were pipetted onto MHA and spread evenly using a sterile cotton swab. The agar plate was incubated at 37 °C for 24 h. MBC is defined as the lowest plant extract concentration that kills 99.9% of the original bacterial inoculum [20], which translates to a maximum of 8 colonies grown on the agar plate.

2.8 Data analysis

The MIC and MBC values are expressed as the mean of three consistent replicates. The total activity of an active extract, expressed as mL/g, is calculated by dividing the quantity of material extracted from one gram of plant material by the MIC value of the extract [21].

3. Results and discussion

The secondary metabolites of the selected medicinal plants were obtained by sequential extraction using solvents of increasing polarity, from the most nonpolar solvent hexane to the most polar solvent water. This is to ensure that a wide polarity range of compounds can be extracted, and to allow the identification of extracts that show a specific antibacterial activity. The total yields for the extracts of *A. esculentus*, *B. alba*, *C. microcarpa*, *C. calamitosum*, and *N. officinale* were 4.39%, 1.85%, 7.72%, 3.77%, and 1.62%, respectively. As for *P. speciosa*, the seed extracts (11.2%) had much higher yield than the pod extracts (3.80%). The yields of individual extracts are shown in Figure 1.

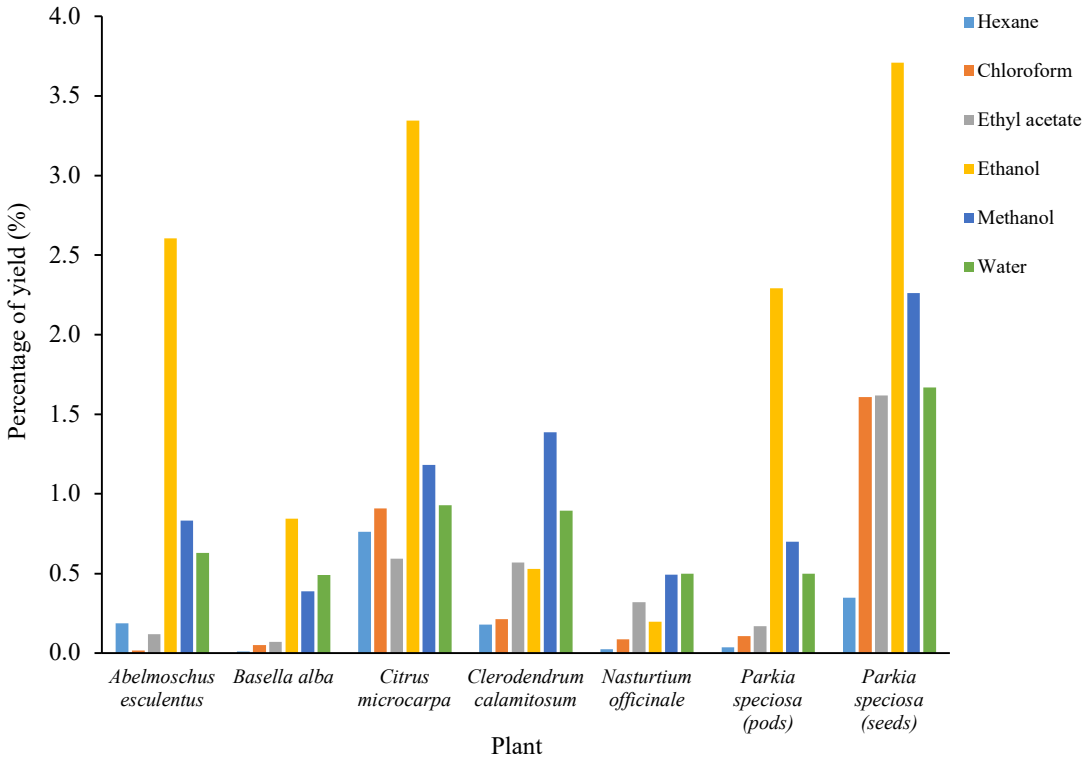

Figure 1 Yields of the extracts of six medicinal plants obtained using sequential solvent extraction

As shown in Table 1, all six medicinal plants possessed antibacterial activity but with different degrees of bacteriostatic and bactericidal activity. With a total of 42 extracts, 32 extracts (76.2%) showed activity in the

MIC assay while only 20 extracts (47.6%) displayed a positive result in the MBC assay. This indicates that the plant extracts possess a broader bacteriostatic activity than bactericidal activity against the five species of bacteria at the concentration range evaluated in the study. The MIC range for the extracts that displayed bacteriostatic activity was 0.02-2.50 mg/mL: this was comparable to the previous studies of plant extracts using the same extraction technique [19,22]. This MIC range was much higher than that of the antibiotic chloramphenicol (MIC: 1-8 µg/mL) as an extract is a mixture of many plant secondary metabolites obtained using a solvent or extractant.

Table 1 Minimum inhibitory concentrations and minimum bactericidal concentrations of medicinal plant extracts versus human pathogenic bacteria.

Extract	Minimum inhibitory concentration (mg/mL)[a]					Minimum bactericidal concentration (mg/mL)[a]				
	B.C.	S.A.	E.C.	K.P.	P.A.	B.C.	S.A.	E.C.	K.P.	P.A.
Abelmoschus esculentus										
Hexane	0.31	0.31	0.63	0.31	0.31	0.31	>2.50	2.50	>2.50	>2.50
Chloroform	0.31	0.31	0.31	0.16	0.16	>2.50	>2.50	>2.50	>2.50	>2.50
Ethyl acetate	0.31	1.25	0.63	0.63	0.31	>2.50	>2.50	2.50	>2.50	>2.50
Ethanol	2.50	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-
Methanol	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Water	>2.50	0.63	>2.50	>2.50	>2.50	-	>2.50	-	-	-
Basella alba										
Hexane	0.16	0.08	0.04	0.04	0.04	0.16	0.08	0.04	0.04	0.04
Chloroform	0.31	0.16	0.16	0.04	0.04	0.31	0.16	0.31	0.04	0.04
Ethyl acetate	0.63	0.31	0.63	0.31	0.63	0.63	0.63	0.63	0.31	0.63
Ethanol	1.25	2.50	2.50	0.63	1.25	1.25	2.50	2.50	0.63	1.25
Methanol	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Water	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Citrus microcarpa										
Hexane	0.31	0.63	1.25	0.31	0.31	>2.50	>2.50	1.25	>2.50	>2.50
Chloroform	0.16	0.63	1.25	0.63	0.31	>2.50	2.50	2.50	>2.50	>2.50
Ethyl acetate	0.31	1.25	2.50	0.31	0.63	>2.50	>2.50	2.50	2.50	>2.50
Ethanol	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Methanol	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Water	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Clerodendrum calamitosum										
Hexane	0.31	0.31	0.31	0.63	0.31	0.31	1.25	0.31	0.63	0.31
Chloroform	1.25	0.63	1.25	0.63	1.25	>2.50	>2.50	>2.50	>2.50	>2.50
Ethyl acetate	0.63	1.25	>2.50	0.31	0.63	>2.50	>2.50	-	>2.50	>2.50
Ethanol	>2.50	0.63	>2.50	>2.50	>2.50	-	>2.50	-	-	-
Methanol	>2.50	2.50	>2.50	>2.50	>2.50	-	>2.50	-	-	-
Water	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Nasturtium officinale										
Hexane	0.16	0.04	0.04	0.08	0.02	0.63	0.04	0.04	0.08	0.02
Chloroform	0.63	0.08	0.04	0.31	0.02	>2.50	0.08	0.04	2.50	0.04
Ethyl acetate	1.25	0.31	0.16	0.63	0.63	2.50	0.63	0.16	1.25	0.63
Ethanol	1.25	0.31	0.31	0.63	0.63	2.50	0.63	0.31	0.63	1.25
Methanol	2.50	2.50	>2.50	2.50	2.50	2.50	>2.50	-	2.50	2.50
Water	>2.50	2.50	>2.50	>2.50	2.50	-	>2.50	-	-	2.50
Parkia speciosa (pods)										
Hexane	0.63	0.63	0.63	0.31	0.31	>2.50	1.25	0.63	0.31	2.50
Chloroform	0.31	1.25	1.25	0.63	0.31	>2.50	>2.50	1.25	>2.50	>2.50
Ethyl acetate	0.31	0.63	1.25	0.63	0.63	>2.50	>2.50	>2.50	>2.50	>2.50
Ethanol	1.25	1.25	1.25	1.25	1.25	>2.50	>2.50	>2.50	>2.50	>2.50
Methanol	2.50	>2.50	>2.50	2.50	2.50	>2.50	-	-	>2.50	>2.50
Water	0.63	1.25	1.25	1.25	1.25	>2.50	>2.50	2.50	>2.50	>2.50
Parkia speciosa (seeds)										
Hexane	1.25	1.25	>2.50	1.25	1.25	>2.50	>2.50	-	>2.50	>2.50
Chloroform	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Ethyl acetate	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Ethanol	1.25	>2.50	>2.50	1.25	1.25	>2.50	-	-	>2.50	>2.50
Methanol	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Water	>2.50	>2.50	>2.50	>2.50	>2.50	-	-	-	-	-
Chloramphenicol[b]	0.002-0.004	0.001-0.004	0.002-0.008	0.001-0.004	0.001-0.004	-	-	-	-	-

[a]The data are shown as the mean of three consistent replicates. [b]positive control. B.C.: *Bacillus cereus* (ATCC 11778™); S.A.: *Staphylococcus aureus* (ATCC 6538™); E.C.: *Escherichia coli* (ATCC 35218™); K.P.: *Klebsiella pneumoniae* (ATCC 13883™); P.A.: *Pseudomonas aeruginosa* (ATCC 27853™). '-': not applicable or not performed.

Among the extracts of *A. esculentus* and with a MIC range of 0.16-1.25 mg/mL, only the hexane, chloroform, and ethyl acetate extracts showed bacteriostatic activity against all bacterial species. However, the extracts concerned showed limited bactericidal activity. The dry fruit of *A. esculentus* has been evaluated for antibacterial activities using disk diffusion method, and the ethanol and methanol extracts were found to be

active against several species of bacteria, including *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa* [6,23]. In contrast, the present study did not detect any significant activity for these two extracts. Besides the difference between fresh fruit and dry fruit used, the discrepancy of results could also be attributed to the sequential extraction used in the present study compared to individual solvent extraction used in the previous studies. Palmitic and stearic acids have been implicated in the antibacterial properties of the water extract of *A. esculentus* [24].

Unlike *A. esculentus*, all extracts of the aerial part of *B. alba*, except water extract, exhibited bacteriostatic (MIC: 0.04-2.50 mg/mL) and bactericidal activities (MBC: 0.04-2.50 mg/mL) against all bacterial species (Table 1). The MIC and MBC values of the extracts has increased from hexane extract to methanol extract, suggesting the nonpolar extracts (hexane and chloroform) of *B. alba* have stronger antibacterial activity than the polar extracts (ethanol and methanol). A literature search revealed contradictory results on the antibacterial properties of *B. alba*. The fractions separated from the methanol extract that was obtained from the dry whole plant of *B. alba* from Bangladesh were reported as being active against Gram-positive bacteria, including *B. cereus* and *S. aureus*, but not active against any of the Gram-negative bacteria studied using disk diffusion method [25]. Sushila et al. [26] reported a much lower MIC range (6.25-12.5 µg/mL) against Gram-positive and Gram-negative bacteria than the present study for the methanol extract of the whole plant of *B. alba* from India. However, no evaluation of MBC for the extract was attempted in the study. Meanwhile, Oyewole et al. [27] reported a MIC range of 50-100 mg/mL for the ethanol extract of the dry leaves from Nigeria against *S. aureus*, *E. coli*, and *P. aeruginosa*. The MBC values for the extract were the same as the reported MIC values, which were 20-40 folds higher than the present study. The methanol extract of the dry leaves from Thailand was found to be inactive against *B. cereus*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *Salmonella typhi* using disk diffusion method [28]. These results may imply that geographical origin, freshness of plant, and plant parts play important roles in determining the antibacterial properties of *B. alba*. Many classes of secondary metabolites such as anthraquinones, saponins, steroids, tannins, and terpenes as well as phytochemicals such as β-sitosterol and lupeol have been detected in the leaves or the whole plant of *B. alba* [26,27]. The nonpolar extracts of *B. alba* warrant further work on isolation and identification of bioactive compounds due to their strong antibacterial activity which is less than 0.1 mg/mL.

For *C. microcarpa*, only the less polar extracts, i.e., hexane, chloroform, and ethyl acetate extracts showed bacteriostatic activity against all bacterial species but with limited bactericidal activities. Nevertheless, the hexane extract produced the highest total activity (56.84 mL/g) in this study against *B. cereus* (Table 2): highlighting the potential of the compounds in this extract as antibacterial agents. Nobiletin, a flavonoid isolated from the dichloromethane extract of the leaves of *C. microcarpa*, is reported to be active against *Bacillus subtilis* and *P. aeruginosa* but inactive against *S. aureus* and *E. coli* [29]. The leaf essential oils of *C. microcarpa*, which contain mainly sesquiterpenoids, have been shown to have antibacterial activity against *B. subtilis, S. aureus, E. coli*, and *P. aeruginosa* [30]. Our results suggested that active compounds other than flavonoids and sesquiterpenoids may also account for the antibacterial activities of the leaves of *C. microcarpa*.

Table 2 Total activity of medicinal plant extracts against human pathogenic bacteria.

Extract	Total activity (mL/g)				
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
Abelmoschus esculentus					
Hexane	6.09	6.09	2.99	6.09	6.09
Chloroform	0.51	0.51	0.51	0.98	0.98
Ethyl acetate	3.83	0.95	1.89	1.89	3.83
Ethanol	10.42	-	-	-	-
Methanol	-	-	-	-	-
Water	-	10.00	-	-	-
Basella alba					
Hexane	0.65	1.31	2.62	2.62	2.62
Chloroform	1.63	3.16	3.16	12.65	12.65
Ethyl acetate	1.14	2.31	1.14	2.31	1.14
Ethanol	6.74	3.37	3.37	13.37	6.74
Methanol	1.55	1.55	1.55	1.55	1.55
Water	-	-	-	-	-
Citrus microcarpa					
Hexane	24.61	12.11	6.10	24.61	24.61
Chloroform	56.84	14.44	7.28	14.44	29.34
Ethyl acetate	19.14	4.75	2.37	19.14	9.42
Ethanol	-	-	-	-	-
Methanol	-	-	-	-	-
Water	-	-	-	-	-
Clerodendrum calamitosum					
Hexane	5.73	5.73	5.73	2.82	5.73
Chloroform	1.72	3.40	1.72	3.40	1.72
Ethyl acetate	9.05	4.56	-	18.39	9.05
Ethanol	-	8.37	-	-	-
Methanol	-	5.55	-	-	-
Water	-	-	-	-	-
Nasturtium officinale					
Hexane	1.58	6.33	6.33	3.17	12.67
Chloroform	1.37	10.77	21.54	2.78	43.07
Ethyl acetate	2.57	10.35	20.06	5.09	5.09
Ethanol	1.58	6.38	6.38	3.14	3.14
Methanol	1.97	1.97	-	1.97	1.97
Water	-	2.00	-	-	2.00
Parkia speciosa (pods)					
Hexane	0.57	0.57	0.57	1.16	1.16
Chloroform	3.42	0.85	0.85	1.68	3.42
Ethyl acetate	5.43	2.67	1.35	2.67	2.67
Ethanol	18.32	18.32	18.32	18.32	18.32
Methanol	2.80	-	-	2.80	2.80
Water	7.94	4.00	4.00	4.00	4.00
Parkia speciosa (seeds)					
Hexane	2.79	2.79	-	2.79	2.79
Chloroform	-	-	-	-	-
Ethyl acetate	-	-	-	-	-
Ethanol	29.66	-	-	29.66	29.66
Methanol	-	-	-	-	-
Water	-	-	-	-	-

The data are shown as the mean of three consistent replicates. '-' denotes not applicable due to the absence of antibacterial activity.

Despite the traditional medicinal uses of *C. calamitosum*, literature shows that the plant has rarely been studied scientifically. The results from this study indicated that the leaves of *C. calamitosum* possessed antibacterial activity against Gram-positive and Gram-negative bacteria. More studies should be done to explore the phytochemical content or other biological activities of this plant.

All extracts of *N. officinale* showed bacteriostatic and bactericidal activities against all bacterial species. Moreover, the hexane extract recorded the lowest MIC and MBC values of 0.02 mg/mL (Table 1) while its chloroform extract produced the second-highest total activity (Table 2), both against *P. aeruginosa*. Our findings corroborated with the positive results reported using disk or well diffusion method [31]. Recently, Thibane et al. [32] studied the petroleum ether, dichloromethane, 70% ethanol, and water extracts of the dry leaves of *N. officinale* from South Africa, and reported a MIC range of 1.56-12.5 mg/mL against *S. aureus*, *E. coli*, and *K. pneumoniae*. The higher range of MIC values compared to the present study are likely due to agro-geographical reasons, type of materials (fresh vs dry), or types of solvent used in the sequential extraction. The results from the present study also reflected the diversity of bioactive secondary metabolites in this plant. The aerial part of *N. officinale* is reported to contain at least 38 metabolites belonging to various classes, i.e., glucosinolates, isothiocyanates, flavonoids, phenolic acids, proanthocyanidins, monoterpenoids, sesquiterpenoids, and saturated fatty acids [14].

The extracts from the seeds and pods of *P. speciosa* had distinctive antibacterial profiles. All pod extracts showed bacteriostatic activity against all five bacterial species (MIC: 0.31-2.50 mg/mL) while for the seed extracts, only hexane and ethanol extracts exhibited bacteriostatic activity against four species and three species of bacteria, respectively (MIC: 1.25 mg/mL), and none of these two extracts were able to kill the bacteria. This study has also demonstrated for the first time that the pods of *P. speciosa* had much stronger antibacterial activity than its seeds, although antibacterial activities have been reported for the chloroform and ethanol extracts of the seeds [17,33] and the 50% ethanol extract of the pods [34] in separate studies. Four cyclic polysulfides (1,2,4,5,7,8-hexathionane, 1,2,4-trihiolane, lenthionine, and 1,2,4,6-tetrathiepane) with antibacterial property have been isolated from the seeds [33]. Besides organosulfur compounds, alkaloids, flavonoids, terpenoids, and phenolic compounds are also present in the seeds of *P. speciosa* [17]. The pods have been reported to contain alkaloids, flavonoids, phenolic compounds, saponins, and tannins [35]. Based on these results, more antibacterial compounds can be anticipated from the pods than the seeds. The pods of *P. speciosa*, which is usually treated as fruit waste, could be explored as a source of bioactive compounds, and this may help to enhance food waste valorization.

4. Conclusion

The findings from this study indicated that the fruits of *A. esculentus*, the pods of *P. speciosa*, the aerial parts of *B. alba* and *N. officinale*, and the leaves of *C. microcarpa* and *C. calamitosum* possessed bacteriostatic and bactericidal activities against human pathogenic bacteria. The antibacterial activity was found to be dependent on the solvents used in the extraction, bacterial species, extract concentrations, and the parts of the plant used. The results of this study form a good basis for the selection of plants for further phytochemical investigations. Since the extracts of *B. alba* and *N. officinale* displayed the broadest bactericidal activity, these two plants can be prioritized for subsequent investigations to identify the bioactive compounds responsible for the antibacterial property. The two plants are regarded as underutilized vegetables in many tropical countries and should be widely promoted for cultivation as a food source with health-promoting activities such as antibacterial property, in addition to their nutritional values. Such an effort will help to contribute to the achievement of the United Nations Sustainable Development Goal 2: Zero Hunger in the future.

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6. References

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