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Phytochemical composition and antibacterial activity of selected medicinal plants in a local community in the Philippines

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Abstract

A survey of medicinal plants in a local community in Guimbal, Iloilo, Philippines identified ten plant species namely, hagonoy (*Chromolaena odorata*), pitogo (*Cycas rumphii*), adgao (*Premna odorata*), labnog (*Ficus leucantatoma*), talus (*Homalomena rubescens*), sinaw-sinaw (*Peperomia pellucida*), palochina (*Senna alata*), badyang (*Alocasia macrorrhizos*); bagacay (*Bambusa vulgaris*) and karupi (*Alpinia* sp). From these plants, two least-studied medicinal plants, *B. vulgaris* and *Alpinia* sp. were determined of their phytochemical composition and antibacterial properties following standard procedures. Aqueous and ethanolic extracts were prepared from these plants and the antibacterial activity of the extracts against two Gram-negative bacteria, *Aeromonas hydrophila* and *Vibrio harveyi*, and a Gram-positive, spore-forming bacterium, *Bacillus albus* were determined using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Phytochemical analyses of *B. vulgaris* and *Alpinia* sp., indicated the presence of alkaloids, carbohydrates, glycosides, phytosterols, flavonoids and phenols and tannins. All extracts of the two medicinal plants inhibited the growth of *A. hydrophila* at a concentration of 0.5 g ml $^{-1}$. The MBC and MIC for *B. vulgaris* aqueous extract and *Alpinia* sp. ethanolic extract against *V. harveyi* showed inhibition at 0.5g ml $^{-1}$ except for the MIC of *B. vulgaris* aqueous extract which was at 0.25 g ml $^{-1}$. There was no inhibition of *B. albus* from all extracts of both medicinal plants. These two medicinal plants can be further explored as potential sources of ingredients for the development of novel antibacterial drugs particularly in inhibiting Gram-negative bacteria.

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Introduction

In many tropical countries, medicinal plants are often used on a regular basis as an alternative or supplement to prescribed medicines that are hard to obtain. With over 13000 plant species present, the Philippines is considered one of the most important biodiversity hotspots in the world (Nuneza *et al.*, 2021). People in many rural areas in the country rely on traditional medicinal plants as remedies for almost all ailments due to their accessibility, availability and cultural acceptability (Hussain *et al.*, 2018). However, the vast knowledge of the economic and medical use of many plants is yet to be discovered for the advancement and development of novelties in drugs.

Natural products or derivatives account for more than a third of all Food and Drug Administration-approved medications and 48.6% of all cancer drugs registered. Medicinal plants are one of the notable sources of natural products. Many of its organs (Sofowara *et al.*, 2013) contain several phytochemicals, such as flavonoids, alkaloids, tannins, and terpenoids, which possess anti-microbial and antioxidant properties (Talib and Mahasneh, 2010). The discoveries on the presence of different bioactive and anti-microbial components of medicinal plants helped researchers track the sources of new and more effective drugs. According to Oladeji (2016), some drugs believed to be obtained from medicinal plants are aspirin, atropine, artemisinin, colchicine, digoxin, ephedrine, morphine and physostigmine.

Moreover, published studies each year helped further recognize a variety of plants as medicinal. This includes botanical surveys that allow people to gain a better understanding of the various plant species that exist in the environment. Plant surveys are also crucial because they serve as a foundation for new discoveries about medicinal plant applications and approved therapeutic actions. A listing prepared by Carag and Buot (2017) from available published literature recorded at least 1000 medicinal plant species and the common ailments each species is utilized for. This checklist is an indication that the Philippines has a rich medicinal flora and practitioners of folk medicine possess extensive knowledge of the medicinal properties of these various plants. However, the rapid land degradation, accelerated forest destructions, loss of biological diversity, access to modern medicine, exposure to modern culture, mobility, and displacement of communities may affect the traditional knowledge and the variety of the medicinal plants that are present in a local community (Cordero and Alejandro, 2021). It is, therefore, urgent to document these data in those local communities before it is totally forgotten. The local community of Camangahan in Guimbal, Iloilo is home to a diverse range of plant species. Some are currently regarded as therapeutic plants, but others have yet to be discovered for their antibacterial properties. However, there is a scarcity of research studies on the various medicinal plants that are present and used in the community. Moreover, their medicinal and antibacterial properties are not well-documented. Hence, these prompted the researchers to conduct a survey of medicinal plants within the locality and to determine the phytochemical composition and antibacterial activities of the least-studied medicinal plants during the survey.

Materials and methods

Study area

The study was conducted in the municipality of Guimbal. This is a coastal municipality that is located in the south-western part of Iloilo province. It is situated between the coordinates of 1250 57' longitude and 100 39' latitude. It is 29 kilometers (18 mi) from Iloilo City and has a total land area of 4,461 hectares (11,020 acres). The municipality consists of 33 barangays (local communities), 22 of which are located outside the town center and 11 are located within the town center. Camangahan, a community that is located outside of the town center, is abundant in medicinal plants, but some of which are unknown.

Data gathering

Due to COVID restrictions and to avoid physical contact, the gathering of information about the medicinal plants that are being used by the local community was done through phone interviews. A total of 30 local informants were interviewed by phone and were only asked to mention the medicinal plants they used and their traditional applications. The names of the participants were kept anonymous and personal information was not acquired. The information gathered was treated with safety and confidentiality.

Collection, enumeration, and description of medicinal plants

Photographic data of uncommon medicinal plants present was taken for proper photo documentation and identification. The samples of these medicinal plants were enumerated and identified using published studies, personal interviews and verification of botanists. Published literature were also used to describe the botanical description, phytochemical properties and medicinal uses of plants.

Preparation of aqueous and ethanolic extracts

Preparation of both aqueous and ethanolic extracts was done following the methods of Gonelimali *et al.* (2018) with modifications. Prior to antibacterial testing, the part of the plant tested was air-dried for 48 hours. The seeds of karupi (*Alpinia sp.*) were utilized by the researchers, while the stems of bagacay (*Bambusa vulgaris*) were used. The identities of these plants were confirmed by the National Museum of Natural History in Manila, Philippines, based on close-up photos of the plant that showed its life habits, the leaves, flowers and fruits that were sent electronically. After air drying, the plant parts were ground using a blender. In a clear container, 100 g of powder from each tested plant material was soaked in 500 ml of distilled water and chilled for 24 hours. Similarly, 100 g powder of each tested plant material was soaked separately in another container with 500 ml of 80% ethanol and chilled for 24 hours.

Liquid extracts obtained were separated from the solid residue by filtration using a cheesecloth. The filtrates were placed into a beaker and dried using a water bath. The residues were weighed, dissolved in 1% normal saline solution (NSS) to a final concentration of 2 g ml^{-1}, which served as the stock solution, and kept at -20^{o}C until use for the analyses.

Phytochemical analysis

In order to determine the phytochemical properties of *Bambusa vulgaris and Alpinia sp.*, the researchers utilized the methods of Khalid *et al.* (2018) and Shaikh and Patil (2020) with slight modifications. The homogenate from the liquid extracts was used for the analysis. The tests for the presence of alkaloids, carbohydrates, glycosides, phytosterols, flavonoids, phenols and tannins were carried out.

Preparation of inoculum

The antibacterial properties of medicinal plant extracts were tested against two Gram-negative bacteria: *Aeromonas hydrophila* and *Vibrio harveyi*, and *Bacillus albus,* a Gram-positive, spore-forming bacterium. The aforementioned broth cultures of these bacteria were obtained from the Biology Laboratory of the University of San Agustin. All bacteria were cultured using Nutrient Broth (NB) with the exception of *V. harveyi*, which was cultured in NA with 1% sodium chloride.

After a 24-h incubation, ten-fold serial dilution of the bacterial solutions was prepared and plated onto Nutrient Agar medium and the bacterial count was determined and expressed as colony-forming units ml^{-1} (CFU ml^{-1})). The bacterial solutions were diluted to obtain a concentration of 1×10^4 CFU ml^{-1} and were utilized for the subsequent antibacterial assays.

Antibacterial assays

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method. Each extract contained four different concentrations: 0.125g ml^{-1} 0.25g ml^{-1}; 0.5g ml^{-1} and 1g ml^{-1}. Fifty microliters (50 µl) of each extract with the specific concentration was pipetted in each well of the 96 well plates with three replicates. Each replicate was added with an equal volume of the bacteria *(A. hydrophila, V. harveyi, B. albus*) at a concentration of 1×10^4 CFU ml^{-1}. The microplate was placed in an incubator at 28-30ºC °C for 18–24 h. The control contained only nutrient broth added with an equal volume of each bacterium. MIC was determined as the lowest concentration of either the aqueous and ethanolic extract, where no visible growth of the bacteria was observed in each well. The visual turbidity of the wells was noted after incubation to confirm the MIC value.

The minimum bactericidal concentration (MBC) was determined by the lowest concentration that inhibited 99.9% of bacterial growth. This was done by streaking individual wells containing the mixtures of the plant extracts and the bacteria onto the Nutrient Agar (NA) plates for *B. albus* and *A. hydrophila*; and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar plates for *V. harveyi*. The plates were incubated for 24 h at 28-30ºC and observed for bacterial growth. The lowest concentration that did not result in bacterial growth was the MBC value for that particular plant extract.

Results

Medicinal plants in Camangahan, Guimbal Iloilo

A total of ten (10) medicinal plants were identified from the locality (Table 1). These included: Hagonoy (*Chromolaena odorata*); Pitogo *(Cycas rumphii);* Adgao; *(Premna odorata);* Labnog *(Ficus leucantatoma Merr.);* Talus (*Homalomena rubescens* (Roxb.) Kunth); Sinaw-sinaw (*Peperomia pellucida*); Palochina (*Senna Alata*); Badyang (*Alocasia macrorrhizos*); Bagacay *(Bambusa vulgaris) and* Karupi (*Alpinia sp*).

Table 1. Enumeration and description of the medicinal plants in Camangahan, Guimbal, Iloilo based on botanical description, phytochemical components and medicinal uses.

Medicinal Plant	Botanical Description	Phytochemical Components	Medicinal Uses
Hagonoy (*Chromolaena odorata*) 	Habit: A perennial shrub that grows up to 3–7 m zones (Vijayaraghavan *et al.* 2017) Leaves: Arrowhead-shaped (6–12 cm in length and 3–7 cm in width) with three veins in pitchfork-shaped appearance Flowers: Have 5–25 tubular florets per head, each 10 mm long that are either white, purple, pink, or blue. Seeds: brown-gray to black in color and is 4–5 mm long (Sirinthipaporn and Jiraungkoorskul, 2017).	Leaves of this plant have been found to be a rich source of flavonoids saponin triterpenoids, tannins, and organic acids. (Vijayaraghavan *et al.*, 2017)	Wound healing, antibacterial, antispasmodic, antiprotozoal, antitrypanosomal, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic, hepatotropic immunomodulatory and anticancer effects (Vijayaraghavan *et al.*, 2017).
Pitogo *(Cycas rumphii)* 	Habit: A small tree or shrub that grows up to 10 m in height with the trunk diameter reaches up to 400 mm. Bark is gray with diamond and rectangular shape. (Khan *et al.* 2011). Leaves: 1.5-2.5 m long, ends with a paired glossy pinnae or a spine 1-3 mm in length Fruit: Sarcotesta has 3-4 mm thickness Seeds: Green-orange color, 4.5-5 cm long, 3-3.5 cm in diameter, flattened-ovoid shape (Hill, 1994).	Cycasin, β-glycosidase; amentoflavone; podocarpus flavone A, 2,3-dihydro amentoflavone; 2,3-dihydro hinoki flavone; isoginkgetin and bilobetin (Khan *et al.*, 2011)	Effective for malignant ulcers, sore throats, wounds healing, piles, boils, itchy skin lesions, nephritic pains, edematous swellings, dizziness, headaches and tuberculosis (Khan *et al.*, 2011).
Adgao *(Premna odorata)* 	Habit: An evergreen small tree or shrub nearly 10 m tall with diameter breast height ranging between 15–30 cm. Leaves: Leaves are opposite, ovate, hairy and green in color, of 7–20 cm in length and 4–13.5 cm in width Flowers: flowers are pale green, yellowish or white that are in inflorescences of 4–15 cm long Fruits: globose drupe-like fruit with fleshy mericarps (Youseff *et al.*, 2021)	Alkaloids, anthraquinone, saponins and steroids (Mollejon and Mollejon, 2019).	Stomachache, headache, phlegm, cough, and tuberculosis (Lirio *et al.*, 2014).
Labnog *(Ficus septica)*	Habit: A dioecious tree that grows 25 m tall and 20 cm in diameter with a smooth, gray bark Leaves: The leaves are smooth and shining, oblong-ovate to elliptic ovate, margin is entire, 10-20 cm in length with sharp point and pointed base.	Tannins, alkaloids, 2-deoxy sugars, quaternary base, and benzopyrone nucleus (Vital *et al.*, 2010)	Used as diuretic, analgesic, laxative, anti-microbial, and antifungal. It is also effective in treating fever, colds, diarrhea, and cough (Jangad and Licardo, 2018; Haryanti *et al.*, 2021)

	Flowers: uniovulate female flowers and male flowers in single or in pairs and are axillary (Conchou 2014). Fruits: 1 mm long with tubercles (Mustaqim 2020) Seeds: Orbicular cotyledons, present in female plants but absent in males. (Conchou *et al.*, 2014).		
Talus (*Homalomena rubescens* (Roxb.) Kunth) 	Habit: A leafy herbaceous plant that grows 10 cm tall Leaves: Green-gray color, triangular, 8-10 cm long, 3-5 cm wide, pinnately netted vein Flowers: Small, gray-brown color, 2-5 inflorescences Fruits: absent Seeds: Ellipsoid or elongate, endosperm copious, embryo axile, significantly costate (Van *et al.*, 2021).	Monoterpene hydrocarbons, sesquiterpenes (Van *et al.*, 2021).	Antibacterial, and antioxidant (Van *et al.*, 2021).
Sinaw-sinaw (*Peperomia pellucida*) 	Habit: A herb that grows branched and upward up to 4 cm. Stems are succulent, round, and 5 mm thick. Spikes are present with green pigment, slender, and 1-6 cm long. Leaves: Heart-shaped with alternate pattern, smooth, and transparent. Flowers: arranged in spike inflorescence about 2-6 cm long, enclosed by round bracts Fruits: Dry, indehiscent, round, 0.5 mm wide Seeds: Dot-like appearance and attached to some fruiting spikes (Ooi *et al.*, 2012).	Secoligans, phytosterols, tetrahydrofuran lignans, steroids, tannins, xanthone glycoside carbohydrates, flavonoids, apiols, and triterpenoids (Ooi *et al.*, 2012).	Used as treatment for gout and arthritis, urinary tract inflammations, constipation, kidney diseases, boils, conjunctivitis, hypertension, tumors, abscesses, breast cancer, convulsions, and lowers cholesterol (Ooi *et al.*, 2012).
Palochina (*Senna Alata*) 	Habit: A shrub that produces flowers, grows 1-4 m tall, proliferating in humid areas. Leaves: The leaves are oblong that consists 5 to 14 leaflet sets, intertwined bracts, and strong petioles that are 2-3 mm long. Flowers: Condensed, bright yellow in color, with 7 stamens, and a ovary Fruit: Thick, 10 x 15 in size, crystal-like pod, brown Seeds: Brown and diamond-shaped (Oladeji, 2020).	Flavonoids, alkaloids, anthraquinone, cannabinoid, phenolics, tannins, terpene, and saponins (Oladeji, 2020).	Used to treat diabetes, typhoid, ringworms, scabies, malaria, asthma, eczema, herpes, blotch, hepatitis, gastroenteritis, syphilis, and gut parasitosis (Oladeji, 2020).
Badyang (*Alocasia macrorrhizos*) 	Habit: An erect perennial, rhizomatous, monoecious plant with short trunk that grows up to 5 m tall Leaves: Large, ovate and can grow 1.8 m long and 1.2 m wide, apex is pointed, cordate base, margins are slightly undulate Flowers: Green to white spathe encloses a greenish spadix, inflorescences are large and form in clusters Fruits: Green to scarlet berries, fleshy, 8 cm long, ellipsoid or ovoid Seeds: reddish, grows on the spadix (Lim, 2015).	Alkaloids, oxalic acid, alocasins A-E, oleic acid, linoleic acid, B-lectins, ascorbic acid, and cyanogenic glycosides (Lim, 2015).	Serve as treatment for toothache, abdominal pains, influenza, diarrhea, malaria, tuberculosis, typhoid fever, headaches, inflammations, rheumatism, diabetes, etc. (Lim, 2015).
Bagacay (*Bambusa vulgaris*) 	Habit: An erect plant that can grow 20 m tall and 10 cm in diameter. Stems are yellowish or yellowish-green Leaves: Leaves have spikelets that are oblong and clustered near the branches of inflorescence, lanceolate, 35 cm long and 4 cm wide Flowers: Light-brown, rare Fruits: Rare or absent due to irregular meiosis Seeds: Produces in large amount in 1-3 years and eventually dies (Zheng *et al.*, 2020).	Carbohydrates, glycosides, saponins, alkaloids, flavonoids, phenolics and tannins, phytosterols, and triterpenoids (Fitri *et al.*, 2020).	Used for kidney diseases, fever, diarrhea, inflammations, measles, gonorrhea, tuberculosis, wounds, and ulcers (Owolabi and Lajide, 2015).

Karupi (*alpinia sp.*) 	Habit: A perennial herb with stems that are 1-2 m tall and 2.5 cm in diameter. Leaves: Oblong-lanceolate, aromatic, root tubers Flowers: The flower has a hollow toothed calyx that often split on one side Fruits: Round, red berries Seeds: obtusely angular (Chouni and Paul, 2018)	Tannins, glycosides, phenols, carbohydrates, monoterpenes, sterols, sesquiterpenes, and flavonoids such as quercetin, kaempferol, alpinin, and galangin (Chouni and Paul, 2018)	Used for fungal skin disorders, antirheumatic, dyspepsia, ulcers, bronchitis, diabetes, obesity, inflammations, diuretic, chest pains, sore throat, and kidney diseases (Chouni and Paul, 2018).

Phytochemical components of B. Vulgaris and Alpinia sp.

Table 2 shows the phytochemical compounds present in both karupi (*Alpinia* sp.) and bagacay (*Bambusa vulgaris). Both plants were positive for alkaloids, carbohydrates, glycosides, phytosterols, flavonoids and phenols and tannins.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentrations (MBC) of various plant extracts

MIC of various extracts against A. hydrophila

Table 3 shows the result of MIC of aqueous and ethanolic extracts of *B. vulgaris* and *Alpinia* sp. against *A. hydrophila*. Turbidity was not observed in all extracts at concentrations of 0.5g ml^{-1} and 1g ml^{-1}, indicating that bacterial growth was inhibited. Thus, the lowest concentration of extracts that can inhibit the growth of bacteria is 0.5 g ml^{-1}.

MBC of various extracts against A. hydrophila

Absence in the growth of *A. hydrophila* was observed in all extracts at concentrations of 0.5g ml^{-1} and 1g ml^{-1}. Thus, the lowest concentration of the extracts that can inhibit the growth of the bacteria is 0.5g/ml (Table 4).

Table 2. Phytochemical compounds present in *B. vulgaris* and *A. graminea*.

Aqueous Sample	Alkaloids	Carbohydrates	Glycosides	Phytosterols	Flavonoids	Phenols and Tannins
B. vulgaris	+	+	+	+	+	+
Alpinia sp.	+	+	+	+	+	+

Positive (+): phytochemical is present; Negative (–): phytochemical is absent.

MIC of various extracts against V. harveyi

The MIC of the aqueous and ethanolic extracts of *B. vulgaris* as well as the aqueous extract of *Alpinia* sp. against *V. harveyi* is 0.25g ml^{-1}. On the other hand, the MIC of the ethanolic extracts of *Alpinia* sp. is 0.5g ml^{-1} (Table 5).

MBC of various extracts against V. harveyi

The MBC of the ethanolic extract of *B. vulgaris* and aqueous extract of *Alpinia* sp. against V. harveyi was 0.25g ml^{-1}. However, it was at 0.5 g ml^{-1} for the aqueous extract of *B. vulgaris* and ethanolic extract of *Alpinia* sp. (Table 6).

Table 3. Minimum Inhibitory Concentrations (MIC) of aqueous (aq.) and ethanolic (eth.) extracts against *A. hydrophila*.

Extract	Concentration			
	1g ml^{-1}	0.5g ml^{-1}	0.25g ml^{-1}	0.125g ml^{-1}
A. *B. vulgaris* (aq.)	-	-	+	+
B. *B. vulgaris* (eth.)	-	-	+	+
C. *Alpinia sp.* (aq.)	-	-	+	+
D. *Alpinia sp.* (eth.)	-	-	+	+

Positive (+): Turbidity indicating growth; Negative (–): No turbidity indicating absence of growth.

MIC of various extracts against B. albus

The turbidity was present in all concentrations of ethanolic and aqueous extracts indicating positive results for bacterial growth. There was no inhibition in all concentrations for *Bacillus albus* (Table 7).

MBC of various extracts against B. albus

The presence of bacterial growth was observed in all extracts. Thus, at all concentrations, there was no inhibition of bacterial growth (Table 8).

Discussion

A total of ten (10) medicinal plants including Hagonoy (*Chromolaena odorata*); Pitogo *(Cycas rumphii);* Adgao; *(Premna odorata);* Labnog *(Ficus septica.);* Talus (*Homalomena rubescens* (Roxb.) Kunth); Sinaw-sinaw (*Peperomia pellucida*); Palochina (*Sena alata*); Badyang (*Alocasia macrorrhizos*); Bagacay *(Bambusa vulgaris) and* Karupi (*Alpinia sp.*) were identified from the local community. The majority of these plants were classified as shrubs, herbs, trees, or erect plants.

Table 4. Minimum Bactericidal Concentrations (MBC) of aqueous (aq.) and ethanolic (eth.) extracts against *A. hydrophila*.

Extract	Concentration			
	1g ml $^{-1}$	0.5g ml $^{-1}$	0.25g ml $^{-1}$	0.125g ml $^{-1}$
A. *B. vulgaris* (aq.)	-	-	+	+
B. *B. vulgaris* (eth.)	-	-	+	+
C. *Alpinia sp.* (aq.)	-	-	+	+
D. *Alpinia sp.* (eth.)	-	-	+	+

Positive (+): Indicating growth; Negative (–): Indicating absence of growth.

Table 5. Minimum Inhibitory Concentrations (MIC) of aqueous (aq.) and ethanolic (eth.) extracts against *V. harveyi.*

Extract	Concentration			
	1g ml $^{-1}$	0.5g ml $^{-1}$	0.25g ml $^{-1}$	0.125g ml $^{-1}$
A. *B. vulgaris* (aq.)	-	-	-	+
B. *B. vulgaris* (eth.)	-	-	-	+
C. *Alpinia sp.* (aq.)	-	-	-	+
D. *Alpinia sp.* (eth.)	-	-	+	+

Positive (+): Turbidity indicating growth; Negative (–): No turbidity indicating absence of growth.

The morphology of the leaves, flowers, fruits, and seeds varies among the ten plants identified. Phytochemicals such as flavonoids, triterpenoids, tannins, alkaloids, anthraquinone, saponins, phytosterols, and phenolics have been found in these plants (Vital *et al.*, 2010; Vijayaraghavan *et al.*, 2017; Chouni and Paul, 2018). In addition, *C. odorata*, *F. septica*, and *H. rubescens* have also been identified to have antibacterial, antifungal and anticancer effects (Vijayaraghavan *et al.*, 2017; Jangad and Licardo, 2018; Haryanti *et al.*, 2021; Van *et al.*, 2021).

Furthermore, *C. odorata*, *F. septica, P. pellucida, S. alata*, *A. macrorrhizos*, and *B. vulgaris* have are regarded as diuretic, analgesic, laxative, anti-inflammatory agent and has been used for treating many illnesses including headache, stomachache, urinary tract infections, tuberculosis and diabetes (Ooi *et al.*, 2012; Vijayaraghavan *et al.*, 2017, Jangad and Licardo, 2018; Haryanti *et al.*, 2021).

Table 6. Minimum Bactericidal Concentrations **(MBC)** of aqueous (aq.) and ethanolic (eth.) extracts against *V. harveyi.*

Extract	Concentration			
	1g ml $^{-1}$	0.5g ml $^{-1}$	0.25g ml $^{-1}$	0.125g ml $^{-1}$
A. *B. vulgaris* (aq.)	-	-	+	+
B. *B. vulgaris* (eth.)	-	-	-	+
C. *Alpinia sp.* (aq.)	-	-	-	+
D. *Alpinia sp.* (eth.)	-	-	+	+

Positive (+): Indicating growth; Negative (–): Indicating absence of growth.

The phytochemical analysis of *B. vulgaris* and *Alpinia sp*. revealed the presence of alkaloids, carbohydrates, glycosides, phytosterols, flavonoids and phenols and tannins. The presence of phytochemicals has also been found in the same genus of *Alpinia sp*. which is *Alpinia officinarum*. It was stressed in the study by Balamurugan *et al.* (2019) that the alcoholic extracts with the use of established screening methods, discovered the presence of phenols, tannins, alkaloids, flavonoids, steroids, and quinones. In the study of Fitri *et al.* (2020), *B. vulgaris* has been reported to contain carbohydrates, glycosides, saponins, alkaloids, flavonoids, phenolics and tannins, phytosterols, and triterpenoids.

Table 7. Minimum Inhibitory Concentrations (MIC) of aqueous (aq.) and ethanolic (eth.) extracts against *B. albus*.

Extract	Concentration			
	1g ml $^{-1}$	0.5g ml $^{-1}$	0.25g ml $^{-1}$	0.125g ml $^{-1}$
A. *B. vulgaris* (aq.)	+	+	+	+
B. *B. vulgaris* (eth.)	+	+	+	+
C. *Alpinia sp*. (aq.)	+	+	+	+
D. *Alpinia sp*. (eth.)	+	+	+	+

Positive (+): Turbidity indicating growth; Negative (–): No turbidity indicating absence of growth.

Both the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results of *B. vulgaris* and *Alpinia sp*. against *A. hydrophila*, a Gram-negative bacterium, indicated minimum inhibition of all extracts at 0.5g ml $^{-1}$. In *V. harveyi*, all extracts except ethanolic *Alpinia sp.* exhibited a MIC at 0.25 g ml $^{-1}$. The MBC for the ethanolic extract of *B. vulgaris* and aqueous extract of *Alpinia sp*. was 0.25g ml $^{-1}$, whereas the aqueous extract of *B. vulgaris* and ethanolic extract of *Alpinia sp*. was at 0.5g ml $^{-1}$. This clearly demonstrates that *B. vulgaris* and *Alpinia sp*. are effective antibacterial agents against these two gram-negative bacteria, *A. hydrophila* and *V. harveyi*.

Previous studies have revealed that bamboo possesses antioxidant, anticancer and anti-microbial activities. Bioactive compounds found in *B. vulgaris,* a bamboo species, include alkaloids, flavonoids, saponins, and tannins that contribute to its antibacterial activity (Tanaka *et al*., 2014). In the previous findings of Zhiang *et al.* (2019), ethanolic extracts from leaves of *B. vulgaris* have been found to exert minimum inhibitory effects against a variety of bacterial species, namely *S. aureus* and *B. subtilis,* at a concentration of 20g ml $^{-1.}$ (Zhiang *et al*., 2019). Additionally, the ethanolic extracts and hot aqueous extracts of *B. vulgaris* showed MIC values ranging at concentrations 31.25 - 125 g ml $^{-1}$ against several gastro-intestinal microorganisms such as *E.coli, K. pneumoniae, P. mirabilis, and S. typhi* in the study of Ogu *et al.* (2011).

The phytochemical compounds present in *Alpinia sp.,* such as tannins, alkaloids, flavonoids and saponins, have been detected to possess antibacterial activity (Rini *et al.* 2018). The same phytochemicals were found to be present in the study which largely contributes to the potent antibacterial activity of the aqueous and ethanolic extracts of *Alpinia sp*. against *A. hydrophila and V. harveyi.* In the study of Oonmetta-aree *et al.* (2006), among the four plants tested, the ethanolic extracts of *Alpinia galanga* demonstrated the highest inhibitory activity against *S. aureus.*

The findings of the study showed the minimum inhibitory concentration (MIC) of the ethanolic extract at 0.325 mg ml $^{-1}$ and the minimum bactericidal concentration (MBC) at 1.3 mg ml $^{-1}$ using the broth diluteion method). However, in the study of Voravuthikunchai *et al.* (2005), aqueous extracts of *A. galanga* did not show any inhibitory activities against *S. aureus.*

Table 8. Minimum Bactericidal Concentrations (MBC) of aqueous (aq.) and ethanolic (eth.) extracts against *B. albus*.

Extract	Concentration			
	1g ml $^{-1}$	0.5g ml $^{-1}$	0.25g ml $^{-1}$	0.125g ml $^{-1}$
A. *B. vulgaris* (aq.)	+	+	+	+
B. *B. vulgaris* (eth.)	+	+	+	+
C. *Alpinia sp.* (aq.)	+	+	+	+
D. *Alpinia sp.* (eth.)	+	+	+	+

Positive (+): Indicating growth; Negative (–): Indicating absence of growth.ive (–): Indicating absence of growth.

The results of MIC and MBC for *B. albus* are not similar to the two Gram-negative bacteria mentioned previously. *B. albus* is a spore-forming, Gram-positive, facultative anaerobe bacterium. The presence of turbidity in all extracts indicates bacterial growth. The reason for this is that spore-forming bacterium is resistant to heat, chemicals, and other agents, making them tough to kill. This explains why extracts obtained from *Alpinia sp*. and *B. vulgaris* are ineffective antibacterial agents against *B. albus*. as these bioactive substances are not able to penetrate the thick cell wall components of the spores. In a related study, *Bacillus subtilis* which is also a Gram-positive spore-forming bacteria, was used as a test organism for determining the antibacterial activity of *B. vulgaris* (Zhiang *et al*., 2019). However, the results indicated that *B. vulgaris* was found to be an effective antibacterial agent at a concentration of 20g ml $^{-1}$. It is most likely that Gram-positive bacteria are inhibited by extracts from medicinal plants at higher doses, and these will be explored in future studies.

Conclusion

The results from this study showed that aqueous and ethanolic extracts from B. *vulgaris* and *Alpinia sp.* contained phytochemical components that possess potent bactericidal activities against Gram-negative bacteria, *A. hydrophila* and *V. harveyi*. These two least-studied medicinal plants can be further explored as potential sources of bioactive substances for the development of novel antibacterial drugs.

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