SCIENCE LETTERS

ISSN: 1675-7785
eISSN: 2682-8626

Available online at
http://myjms.mohe.gov.my/index.php/sl

Science Letters 18(2) 2024, 92 – 107.

Science Letters

Antimicrobial Screening of Thirteen Aqueous Plant Extracts against Selected Pathogenic Fish Bacteria

Azirah Akbar Ali[1], Zary Shariman Yahaya[1], Ainil Hawa Jasni[2], Ismail Muhamad Fareez[3,4]*

[1]*School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia*
[2]*Department of Science in Engineering, Faculty of Engineering, International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur, Malaysia*
[3]*School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam Campus, 40450 Shah Alam, Selangor, Malaysia*
[4]*Collaborative Drug Discovery Research Group (CDDR), Faculty of Pharmacy, University Teknologi MARA (UiTM) Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia*

ARTICLE INFO

Article history:
Received 12 March 2024
Revised 25 May 2024
Accepted 5 June 2024
Online first
Published 24 June 2024

Keywords:
Fish disease,
plant extracts,
pathogens,
inhibition,
aquaculture

DOI:
10.24191/sl.v18i2.27019

ABSTRACT

Plants produce bioactive compounds with anti-bacterial properties, making them a viable alternative to antibiotic use in aquatic disease management. However, studies on using aqueous plant extracts in aquaculture as biocontrol agents are still limited. In this study, the anti-bacterial properties of 13 plant extracts, prepared using green and environmentally friendly hot water extraction techniques, were screened against three pathogenic fish bacteria, namely *Aeromonas hydrophila, Streptococcus agalactiae*, and *Staphylococcus xylosus*. Interestingly, the findings revealed that out of 13 extracts, only three plants, *Jacaranda filicifolia, Tamarindus indica*, and *Samanae saman*, demonstrated significantly high potency (as low as 50 mg) in inhibiting these bacteria, as determined by the broth dilution method. By means of the well diffusion method, these three plant extracts exhibited considerably high antimicrobial activity, with a strong inhibition (9-15mm) against all three fish pathogens except *S. saman* with little inhibition on *A. hydrophila* (2mm). The phytochemical test in the selected plants revealed that the extracts of *J. filicifolia* had a high concentration of alkaloids, tannin, and triterpenes. The gas chromatography and mass spectrometry study (GC-MS) indicated a high concentration of hydroquinone (43.16%). In comparison, the HPLC results of *J. filicifolia* indicated the presence of a good content of sucrose (15.17%), galactose (13.46%), glucose (33.06%), and fructose oligosaccharides (38.31%). In general, *J. filicifolia* could be used as an alternative antimicrobial agent for disease management in aquaculture.

INTRODUCTION

Like humans and other animals, fish are prone to diseases. Many fish diseases can be identified by their listless behaviour or skin spots. Meanwhile, some bacterial-related fish diseases can be fatal, and this situation can seriously impact aquaculture. For example, a Gram-negative facultative anaerobic bacterium, *Aeromonas hydrophila*, not only causes internal bleeding in freshwater fish but also leads to severe gastroenteritis, cellulitis, and necrotizing fasciitis in transmission to humans, especially children, or people with compromised immune systems or growth problems [1]. Furthermore, fish diseases caused by β-haemolytic bacteria named *Streptococcus agalactiae* isolated in aquatic animal species can cause meningoencephalitis, sepsis, and cases of unexpected death without symptoms in freshwater fish such as

* Corresponding author. *E-mail address*: fareezismail@uitm.edu.my

tilapia [2]. Another primary pathogen in human medicine is *Staphylococcus xylosus*, commonly found in leafy vegetables and derived from fish and fish products due to poor sanitation, careless handling, and poor personal hygiene [3]. Another primary pathogen in human medicine is *Staphylococcus xylosus*, commonly found in leafy vegetables and derived from fish and fish products due to poor sanitation, careless handling, and poor personal hygiene [3].

This situation can have a serious impact on aquaculture. For example, a Gram-negative facultative anaerobic bacterium, *Aeromonas hydrophila*, not only causes internal bleeding in freshwater fish but also leads to severe gastroenteritis, cellulitis, and necrotizing fasciitis in transmission to humans, especially children, or people with compromised immune systems or growth problems [1]. According to Pulpipat et al. [2], fish diseases causing meningoencephalitis, sepsis, and unexpected death without symptoms can also be caused by β-haemolytic bacteria named *Streptococcus agalactiae* which has been isolated from aquatic animal species. Another primary pathogen in human medicine is *Staphylococcus xylosus*, commonly found in leafy vegetables and derived from fish and fish products due to poor sanitation, careless handling, and poor personal hygiene [3].

Antibiotics and synthetic drugs, such as chloramine, formalin, and tetracycline, are commonly used in fish farming as one of the most simple and effective ways to control aquacultural diseases. However, it was recently found that its use in aquaculture contributes to adverse side effects in fish, shellfish, and even humans. It is currently among the biggest risks to public health [4]. Several nations have banned certain antibiotics after many years because of consumer concerns about their potential health effects. European countries and the United States of America (USA) are among the first countries to ban the use of antibiotics in aquaculture. Antibiotics can accumulate as residues in fishpond or animal tissue and thus lead to uncontrolled growth of antibiotic-resistant pathogenic bacterial strains that can later infect humans [5]. Therefore, an alternative way to replace these chemicals with natural products in monitoring aquaculture diseases is urgently required. Recently, the application of medicinal plants, recognized as rich sources of antimicrobial agents, to control waterborne diseases has attracted much interest in aquaculture, as it has been shown to improve fish immunity [6].

Numerous medicinal plants have been employed in aquaculture techniques up to this point to stop the spread of illnesses within the fish farm. These plants are rich in secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols, and quinones. There are several reports on the antimicrobial activity of different herbal extracts. For example, *Azadirachta indica* is one of aquaculture's first and most commonly used plant extracts to control fish disease due to its effective role in boosting immunity against specific fish diseases and reducing infection [7, 8]. Due to its minimal side effects and low cost, using medicinal plants with such anti-bacterial properties may be an ideal and effective alternative to antibiotics [9, 10]. The selection of potent plants and solvent systems for extraction plays an important role in recovering biomolecules with desirable properties.

For the extraction of plants or herbs, particularly for aquaculture use, one of the keys is that all extraction solvents or chemicals should not have toxic or strong interfering effects on living cells, animals, and humans. Water is the universal solvent that penetrates the plant material, releasing the phytochemicals within those cells. Hot water extracts tend to be more effective antimicrobial agents than cold water extracts due to differences in constituents' extraction or the bioactive fractions' heat sensitivity. Biologically active compounds were easily extracted and reacted at higher temperatures [11]. However, the degree of inhibition of each herb against a variety of bacterial species was different, and this may be caused by the variation of active compounds in herbs that acted to different degrees of inhibition [12].

https://doi.org/10.24191/sl.v18i2.27019

Given those above, the purpose of this study was to ascertain the minimum inhibitory concentration by employing the agar well diffusion method and the broth dilution assay to assess the effectiveness of a few chosen aqueous plant extracts against three pathogenic fish bacteria, *A. hydrophila*, *S. agalactiae*, and *S. xylosus*. Based on the results, phytochemical test analysis was performed in selected plant extracts. The active compounds and polysaccharides were determined by gas chromatography, mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC) analysis.

EXPERIMENTAL

Plant collection and extraction preparation

The plants used in this study consisted of 13 species presented in Table 1. The photograph of each plant species is provided in Supplementary S1. The selection of plants for the screening process was based on random sampling from the University of Sains Malaysia (USM), Penang Campus, Malaysia. The plants are identified and confirmed as species by the Herbarium Centre, USM. Only young leaves were selected for the experiment. The leaves were thoroughly washed under running tap water and dried using a hot air furnace at 40 °C. The leaves were then finely ground to a powder using an electric mill (MX-335, Panasonic, Kuala Lumpur, Malaysia). The powdered products were placed in airtight containers for later use.

Table 1. List of the plants used in the present study

Herbarium number	Samples	Family	Common Name	Growth habit
11354	*Senna spectabilis*	Fabaceae	Kasia Kuning	Tree
11355	*Jacaranda filicifolia*	Bignoniaceae	Jambul Merak	Tree
11356	*Samanae saman*	Fabaceae	Pukul Lima	Tree
11357	*Tamarindus indica*	Leguminosae	Asam Jawa	Tree
11358	*Carica papaya*	Caricaceae	Betik	Shrub or small tree
11359	*Andira inermis*	Leguminosae	Brown Heart	Tree
11360	*Morinda elliptica*	Rubiaceae	Mengkudu kecil	Tree
11361	*Coleus aromaticus benth*	Labiataea	Lemuju	Shrub or small tree
11362	*Citrus hystrix*	Rutaceae	Limau Purut	Tree
11363	*Millettia pinnata*	Fabaceae	Mempari	Tree
11364	*Cymbopogon nardus*	Poaceae	Serai Wangi	Grasses
11365	*Cymbopogon citratus*	Poaceae	Serai	Grasses
11366	*Polyalthia longifolia*	Annonaceae	Asoka	Tree

Hot Water Extraction of plant leaf powder in hot water

A total of 60 g of each powdered plant material was mixed with 300 ml of purified water and heated by a water bath at 70 °C for 6 h. The concentrated extracts were evaporated to dryness in an oven at 40 °C for approximately 48 h. The extracts obtained were kept at 4 °C until necessary.

https://doi.org/10.24191/sl.v18i2.27019

Anti-bacterial activity of plant extracts

Test microorganisms

Three species of bacteria, *A. hydrophila, S. xylosus,* and *S. agalactiae*, have been derived from the stock culture of the National Fish Health Research Centre (NaFish) Penang, Malaysia. The bacterium was kept at 4 °C on the nutrient agar slants until it was required as a working crop and at 15% glycerol at -20 °C for long-term preservation.

Broth Dilution Assay

Out of 13 extracts, three plants were selected for further analysis. MIC is characterized as the lowest concentration of the antimicrobial agent that inhibits microbial growth after 24 h of incubation. The test was monitored using the micro-dilution method. Exactly 95 µl of MH broth, 5 µl of bacterial suspension, and 100 µL of plant extract solution (10 mg/ml) were applied to each well. Meanwhile, 95 µL of MH broth, 5 µL of bacterial suspension, and 100 µL of sterile distilled water were used for negative power. The microtiter plates were then incubated at 30 °C for 24 hours. MIC was determined by adding 40 µl of 0.2 mg/mL of INT to each well and incubating at 30 ° C for 30 min. A pink or red color indicated the growth of bacteria, and clear or colorless wells indicated the inhibition of bacteria growth by water extract. All assays were performed independently three times in triplicate.

Agar Well Diffusion Assay

The well diffusion method was performed to determine the relative antimicrobial effects of the plant extracts. The bacterial suspension (0.1 mL) containing 10^8 CFU/ml was similarly dispersed using a sterile cotton swab. In a laminar flow hood, the agar plates were allowed to dry. Standard ciprofloxacin (30 µg/disc) was used as a positive control, and blank discs were used as a negative control. Two holes were made on each agar plate using a sterile cork borer tool (6 mm in diameter). Approximately 20 µL of plant extracts were filled in one hole, and the second was filled with sterile distilled water as a control. The plates were incubated at 30 ° C for 24 h. Three replicates were made using three Petri dishes of each extract. After incubation, the inhibition zones around the holes were tested to detect antimicrobial activity. All assays were performed independently three times in triplicate.

Phytochemical test of selected leaf extracts

A chemical test analysis was carried out to assess the possible phytochemical constituents present in the best crude plant extracts selected based on the MIC and well diffusion tests. Different chemical methods were used to identify the presence of alkaloids, saponins, flavonoids, tannins, polyphenolics, and triterpenes or steroids. These tests were carried out according to the methods mentioned by Sofowora [13] and Tiwari et al. [14].

The GC-MC content analysis of the selected plant leaf was subsequently performed according to Khattab et al. [15]. Generally, 10 mg of crude extracts were dissolved in 1 ml of absolute alcohol, and the solution was mixed well using a vortex machine. Lastly, the solution was filter sterilized using 0.2 µm syringe filters. The GC–MS-QP2010 instrument (Shidmazu Co. Ltd., Tokyo, Japan) was performed using the BP X5 (SGE) column (30 m x 0.250 mm x 0.25 µm, L x d x H) column and helium as the carrier gas. The inlet temperatures of the instruments were set at 280 °C, column initial temperature 70 °C (hold time: 2 min), ramp temperature 20 °C, and final temperature 280 °C (Final time: 20 min).

https://doi.org/10.24191/sl.v18i2.27019

Plant Polysaccharides Content of plant polysaccharides in selected leaf extracts

One gram of plant powder was mixed with 10 mL of sterile distilled water to identify immune boosting polysaccharides in the plant extracts. One volume of hexane was added to the solution and mixed well. A two-layer formation was formed, and the water layer was collected and washed with one volume of butanol three times. The final solutions were frozen and dried, and the plant polysaccharide samples were stored at -20 ºC until further analysis. HPLC (600E System, Waters, MA, USA) was performed using the Sugar Pack column to identify the polysaccharide compounds in these three plant extracts. Briefly, the equipment was set at a flow rate of 0.5 mL/min (636 psi, 90 °C) using a refractive index (RI) detector.

RESULT AND DISCUSSION

Determination of the antimicrobial activity of different aqueous plant extracts

In this study, 13 aqueous plant extracts were qualitatively screened for their inhibitory actions against three pathogenic bacteria, *A. hydrophila, S. xylosus*, and *S. agalactiae* (Table 2), of which three were selected for further analysis. The antimicrobial activities of the three plant extracts, namely *T. indica*, *J. filicifolia, and S. saman*, were further screened using the well diffusion method and MIC analysis. Although *P.longifolia, S. spectabilis, and A. inermis* indicated some inhibition against *S. xylosus* and *Citrus hystrix* inhibited *S. agalactiae* (Table 2), they are not selected for further studies due to their high selectivity in their inhibitory action against the pathogens tested. In the well diffusion study, the appearance of clear zones around the well indicated antimicrobial activities.

Table 2. Qualitative analysis of antimicrobial activities of thirteen plant extracts against three pathogenic bacteria.

Plant extracts*	**Bacteria suspension (10^8 CFU/ml)**		
	Aeromonas hydrophila	***Streptococcus agalactiae***	***Staphylococcus xylosus***
Senna spectabilis	-	-	+
Jacaranda filicifolia	+	+	+
Samanae saman	-	+	+
Tamarindus indica	+	+	+
Carica papaya	-	-	-
Andira inermis	-	-	+
Morinda elliptica	-	-	-
Coleus aromaticus Benth	-	-	-
Citrus hystrix	-	+	-
Millettia pinnata	-	-	-
Cymbopogon nardus	-	-	-
Cymbopogon citratus	-	-	-
Polyalthia longifolia	-	-	+

*Initial concentration of 50 mg/ml

The antimicrobial properties of the selected plant extracts were further tested against the three pathogenic bacteria using the well diffusion method (Table 3). *J. filicifolia* demonstrated significant

https://doi.org/10.24191/sl.v18i2.27019

antimicrobial activity ($p < 0.05$) against three fish pathogens with a strong inhibition zone in the 10-14 mm range. However, *S. saman* demonstrated anti-bacterial activity against gram-positive bacteria only with an inhibition zone for *S. agalactiae* and *S. xylosus* of at least 12 and 15 mm, respectively. For *T. indica*, activities were detected in *A. hydrophila* and *S. xylosus* with a low-range (9-10 mm) inhibition region.

Table 3. Inhibition activities of three plant extracts against three pathogenic bacteria

Bacteria	**Zone Inhibition (mm)**			
	Aqueous Extracts			**Positive control**
	Tamarindus indica	***Jacaranda filicifolia***	***Samanae saman***	
Aeromonas hydrophila	10.10 ± 0.1^{a}	11.10 ± 0.1^{a}	2.20 ± 0.1^{b}	27.10 ± 0.3^{c}
Streptococcus agalactiae	9.20 ± 0.2^{a}	10.10 ± 0.1^{b}	12.25 ± 0.3^{c}	30.10 ± 0.1^{d}
Staphylococcus xylosus	9.15 ± 0.1^{a}	14.20 ± 0.1^{b}	15.1 ± 0.2^{b}	31.10 ± 0.1^{c}

[a-d] different superscript letters denote that the observed inhibition is significantly different when compared to each respective treatment at a significance level of 0.05

Based on Table 4, the MIC test demonstrated that all three plant extracts inhibited *S. agalactiae and S. xylosu*s, with *S. saman* extracts displaying a relatively more potent inhibition against these two gramme-negative bacteria at a concentration of 1.25 mg/ml and 0.625 mg/mL, respectively, compared to *T. indica* (2.50 mg/ml) and *J. filicifolia* (2.50 mg/ml). Furthermore, *A. hydrophilla*, a gram-positive bacterium, was inhibited by *T. indica* and *J. filicifolia* at concentrations of 5 mg/ml and 2.5 mg/ml. *J. filicifolia* is considered superior and relatively potent in inhibiting the growth of a wider spectrum of pathogenic bacteria compared to *T. indica* and *S. saman*.

Table 4. Minimum Inhibitory Concentration (MIC) values of three plant extracts against three pathogenic bacteria

Bacteria	**Minimum inhibitory concentration (MIC) (mg/ml)**			
	Aqueous Extracts			**Positive control**
	Tamarindus indica	***Jacaranda filicifolia***	***Samanae saman***	
Aeromonas hydrophila	5.00	2.50	-	0.00044
Streptococcus agalactiae	2.50	2.50	1.25	0.00098
Staphylococcus xylosus	2.50	2.50	0.63	0.00096

Phytochemical analysis of selected leaf extracts

A phytochemical test analysis was performed for these three plant extracts, which is presented in Table 5. Extracts of *J. filicifolia* had a high concentration of alkaloids, tannin, and triterpenes. However, the *T. indica* extracts showed a medium occurrence of saponins, flavonoids, and triterpenes and a weak concentration of tannins. Lastly, *S. saman* had a medium tannin concentration and a low concentration of alkaloids, flavonoids, and steroids. Gas chromatography and mass spectrometry (GC-MS) analysis were also performed to determine the active compound in three plant extracts (Table 6). The results showed that *J. filicifolia* had major compounds: hydroquinone (43.16%) and 1, 4-cyclohexanedione (34.91%). *T. indica* showed the presence of 4-O-methylmannose (39.98%), hydroquinone (29.45%), and 1, 2-benzenediol (15.74%). The same compounds were found in *S. saman* extracts, although at low concentrations: 1, 2-benzenediol (10.11%), and 4-O-methyl mannose (26.89%).

https://doi.org/10.24191/sl.v18i2.27019

Table 5: Phytochemical content analysis of selected plant leaf

Tests	**Plants**		
	J. filicifolia	***T. indica***	***S. saman***
Alkaloids	3+	-	1+
Saponins	-	2+	-
Flavonoids	-	2+	1+
Tannins and Polyphenolic	3+(CT)	1+(CT)	2+(CT)
Triterpenes/steroids	3+ (T)	2+(T)	Steroid

Note: 1+ weak color, 2+ mild color, 3+ strong color, CT- Condensed tannin, T- Triterpenes

Identification of plant polysaccharide compounds.

Plant polysaccharide compounds were identified by high-performance liquid chromatography (HPLC). Table 6 shows the results of polysaccharides in three types of plants. The crude plant extracts were subjected to a method to break down the carbohydrate compound into a single monosaccharide molecule. From the results, the crude extracts of *J. filicifolia* showed the presence of sucrose (15.17%), glucose (33.06%), galactose (13.46%), and fructose (38.31%). For the crude extract of *T. indica, sugar polysaccharides,* such as 2-deoxyglucose and fructose, were present with an area percentage of 82.48% and 17.52%, respectively. Lastly, the *S. saman* crude extract showed the presence of sucrose, 2-deoxyglucose, and fructose compounds with an area percentage of 24.18%, 62.06%, and 13.76%, respectively.

Plant extracts effectively prevent fish diseases in aquaculture as an alternative to antibiotic use. Many researchers have investigated plant extracts to control bacterial diseases in fish cultures. A study conducted by Murthy and Kiran [16] evidenced the anti-bacterial activity of the aqueous extract of three medicinal plants, *Azadirachta indica*, *Solanum torvum*, and *Curcuma longa* (rhizome), against the *in vitro* growth of *A. hydrophila,* previously isolated from infected freshwater fish, *Channa striata.* Another study by Kamble et al. [17] demonstrated that the aqueous extract of Moringa oleifera leaves has higher antimicrobial activity with a 13.1 mm inhibition zone compared to the Aegle marmelos leaf extracts with a 7.9 mm inhibition zone when assessed against *S. agalactiae*. This research highlights the potential of some plant extracts with anti-bacterial properties against fish pathogens that benefit the aquaculture and fisheries industries.

https://doi.org/10.24191/sl.v18i2.27019

Table 5. Quantitative estimation of active compounds found in the *Jacaranda filicifolia* plant extract.

Name of Compound	Molecular Formula	Molecular Weight	*Jacaranda filicifolia*		*Tamarindus indica*		*Samanae saman*	
			RT (min)	Peak Area %	RT (min)	Peak Area %	RT (min)	Peak Area %
1,4-Cyclohexanedione	$C_6H_8O_2$	112	6.125	34.91				
Cyclohexanone, 4-hydroxy-	$C_6H_{10}O_2$	114	6.470	1.37				
1,2-Benzenediol	$C_6H_6O_2$	110	6.974	2.36	6.959	15.74	6.988	10.11
Benzofuran, 2,3-dihydro-	C_8H_8O	120	7.187	2.10			7.197	2.18
Acetic acid, 4-oxocyclohexyl ester	$C_8H_{12}O_3$	156	7.345	0.56				
Hydroquinone	C_6H_6O	110	7.638	43.16	7.633	29.45	7.679	4.78
Resorcinol	$C_6H_6O_2$	110	7.715	4.54				
Guaiacol<4-vinyl->	$C_9H_{10}O_2$	150	8.015	1.23	8.015	1.255	8.018	2.82
1,4-Benzenediol, 2-methyl-	$C_7H_8O_2$	124	8.239	3.23	7.772	2.482	7.791	0.90
1,6-Cyclodecadiene, 1-methyl-5-methylene-8-	$C_{15}H_{24}$	204	8.494	0.19				
Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	8.653	0.29				
1-Butanamine, N-(2-furanylmethylene)-3-methyl-	$C_{10}H_{15}NO$	165	8.724	0.18				
Caryophyllene	$C_{15}H_{24}$	204	8.872	0.17	8.876	0.268		0.22
2-Chloropropionic acid, decyl ester	$C_{13}H_{25}ClO_2$	248	9.257	1.06				
Undecanoic acid, 10-methyl-, methyl ester	$C_{13}H_{26}O_2$	214	9.388	0.10				
D-Allose	$C_6H_{12}O_6$	180	9.456	2.53	9.456	2.253	9.495	1.65
Benzeneacetic acid, 2,5-dihydroxy-	$C_8H_8O_4$	168	10.120	0.98				
Diphenyl sulfone	$C_{12}H_{10}O_2S$	218	12.284	0.95	12.287	0.654		
Phenol,2-methoxy	$C_7H_8O_2$	124			6.006	1.442		
1,2-Benzenediol, 4-methyl	$C_7H_8O_2$	124			7.772	1.825		
Phenol, 2,6-dimethoxy	$C_8H_{10}O_3$	154			8.295	0.322	8.296	0.37
4-Ethylcatechol	$C_8H_{10}O_2$	138			8.509	2.359		
Benzene,1,2,3-trimethoxy-5-methyl-	$C_{10}H_{14}O_3$	182			9.517	0.165		
4-O-Methylmannose	$C_7H_{14}O_6$	194			10.743	39.98	9.876	26.89
1,4-Benzenediol, 2-methoxy-	$C_7H_8O_3$	140					7.604	1.01
Sucrose	$C_{12}H_{22}O_{11}$	342					9.048	1.86
7-Decen-2-one	$C_{10}H_{18}O$	154					9.136	0.83
Limonene oxide <trans->	$C_{10}H_{16}O$	152					9.179	0.66
2-Methyl-4-pyridinamine 1-oxide	$C_6H_8N_2O$	124					10.234	0.42
Z-5,17-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308					10.373	0.23
Uric acid	$C_5H_4N_4O_3$	168					10.996	1.27
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	$C_{11}H_{18}N_2O_2$	210					12.209	1.81
Cis-11-Hexadecenal	$C_{16}H_{30}O$	238					12.470	0.86
9-Hexadecyn-1-ol	$C_{16}H_{30}O$	238					12.519	0.49

https://doi.org/10.24191/sl.v18i2.27019

SCIENCE LETTERS

ISSN: 1675-7785
eISSN: 2682-8626

Available online at
http://myjms.mohe.gov.my/index.php/sl

Science Letters 180(2) 2024, 79 – X.

Science Letters

Table 6: Quantitative estimation of polysaccharides found in three types of plant extract

Types of plant	Types of polysaccharides	Area %
Jacaranda filicifolia	Sucrose	15.17
	Glucose	33.06
	Galactose	13.46
	Fructose	38.31
	Total	100
Tamarindus indica	2-deoxy-glucose	82.48
	Fructose	17.52
	Total	100
Samanae saman	Sucrose	24.18
	2-deoxy-glucose	62.06
	Fructose	13.76
	Total	100

Generally, the aqueous extracts possessed much lower potencies than the corresponding organic extracts. However, some plants are more potent as aqueous extracts than organic extracts [18]. Due to its higher polarity relative to chloroform and ethanol, water effortlessly infiltrates the intracellular matrix of the mushroom cell wall [19]. Meanwhile, applying hot-water extraction techniques was more efficient than the cold-water extraction method. However, it could lead to differences in the extracted constituent due to the heat sensitivity and polarity of the bioactive fractions [20]. Some proteins or potentially beneficial antimicrobial metabolites may be denatured. A qualitative analysis by Gebreyohannes et al. [19] revealed that *A. nilotica* extract obtained via cold extraction showed the presence of anthraquinones, terpenoids, saponins, flavonoids, tannins, and cardiac glycosides. Conversely, the extract obtained through the hot extraction method did not exhibit the presence of saponins but slightly increased protein amino acid content [21]. Nevertheless, the choice of extraction method can also be influenced by the characteristics of the plant material itself. Some plant materials may be better suited for hot water extraction due to the heat-stable nature of their active compounds. In contrast, others may be more effectively extracted using the gentle approach of cold-water extraction. Therefore, hot-water extraction techniques with an optimal temperature and extended period used in this study may enhance the extraction yield of the active components of the aqueous extracts with antimicrobial potential [22].

In the present study, the antimicrobial properties of 13 plant extracts were tested against three pathogenic bacteria, *A. hydrophila, S. xylosus,* and *S. agalactiae,* using the Well Diffusion method. Three showed promising anti-bacterial properties, and *J. filicifolia* generally demonstrated strong growth inhibition against all pathogens. On the other hand, *T. indica* only showed anti-bacterial activity against Gram-negative bacteria, while *S. saman* showed a better antimicrobial property against *S. xylosus* than other extracts. Typically, these plant extracts were more effective against Gram-positive bacteria than against Gramme-negative bacteria, which was explained by the antibiofilm activity against gramme-positive bacteria producing biofilms such as *S. xylosus* and *S. agalactiae* [23]. In another study, hydroalcoholic extracts of *J. puberula* leaves that have anti-bacterial activity against *S. aureus* showed bactericidal activity at 100 mg/mL [24]. Furthermore, research by Arulpriya et al. [25] using an aqueous and chloroform extract of *S. saman* showed anti-bacterial activity against Staphylococcus spp. Pathogens. Meanwhile, a study by Adeniyi et al. [26] reported that *T. indica* extracts have significantly inhibited *A. hydrophila* pathogens.

Lower MIC values were obtained from the aqueous extracts of *T. indica* against *A. hydrophila* and *S. aureus*. The anti-bacterial properties of *S. saman* plant extract have been reported in chicken patties to reduce microbial contamination [27]. Very limited studies have been done on the antimicrobial activity of *S. saman* and *J. filicifolia* plant extracts against fish pathogens.

Phytochemical constituents play an important antimicrobial role against a wide range of bacterial spectrum. It can be divided into several groups, such as alkaloids, saponins, flavonoids, tannins, triterpenes, or steroids [28]. The present study performed the phytochemical test on three plant extracts: *J. filicifolia*, *T. indica*, and *S. saman*. Extracts of *J. filicifolia* had a high concentration of alkaloids, tannin, and triterpenes. Meanwhile, the *T. indica* extracts showed a medium occurrence of saponins, flavonoids, and triterpenes and a weak concentration of tannins. Lastly, *S. saman* had a medium tannin concentration and a low concentration of alkaloids, flavonoids, and steroids. More research needs to be done on the phytochemical constituents of the aqueous extracts of these plants. One study was conducted by Prasad et al. [29], who found that *S. saman* exhibited activity against all the organisms tested. The growth of *E. coli* was inhibited at a concentration of five mg/ml, while a higher concentration of 10 mg/mL was required to inhibit the growth of *S. Aureus* and *C. Albicans*. Phytochemical screening of the plant revealed the presence of tannins, flavonoids, saponins, steroids, cardiac glycosides, and terpenoids. Additionally, isoquinoline alkaloids represent one of the larger groups and more interesting secondary metabolites of plants that have the potential to enhance and act as growth promoters [30]. Many alkaloids show obvious pharmacological activity and physiological effects, making them valuable as medicines. Thalkaloidds compound was the main anti-bacterial compound in the plant extracts. The anti-bacterial activity of *Datura metal* plant extracts could be due to various phytochemical constituents such as flavonoids, tannins, saponins, alkaloids, and sterols [31]. The flavonoid group is known for its anti-bacterial, anti- inflammatory, antiviral, antineoplastic, and antithrombotic properties [32].

The antimicrobial activities of tannins are then well documented. Tannins are reported to have various physiological effects, such as anti-irritant, antimicrobial, and anti-parasitic effects. The highest anti-bacterial activities have occurred due to a high tannin content [33]. Tannins are also known to react with proteins to provide the typical tanning effects, which are very important in treating inflammation or ulcerative tissue [34]. Other groups of phytochemicals are saponin and terpenoids. This group of secondary plant metabolites has derived its name from its ability to form stable soap-like foams in aqueous solutions; it is reported to possess various beneficial properties for health [35]. These molecules are reported to have commercial value and may be used as drugs and adjuvants. Saponins, found in plants, can enhance the effectiveness and selectivity of anticancer drugs by increasing the sensitivity of chemoresistant tumor cells to chemotherapeutic agents [36]. Meanwhile, triterpenoid saponins show potential as antiviral, adjuvant, hemolytic, cytotoxic, and anti-angiogenic agents, with potential for treating COVID-19 patients [37]. Terpenoids are active against bacteria, viruses, fungi, and protozoa. Food scientists have found that terpenoids in essential oils were useful in controlling *Listeria monocytogenes* [38]. Triterpenoids are present in a wide range of plants used in traditional medicine and are known to have antitumor properties [39].

This study used gas chromatography and mass spectrometry (GC-MS) to analyze all tested plant extracts. GC-MS analysis is an interesting tool for evaluating several active biocomponents in medicinal plants. In the present study, the chemical profile of the selected plant extracts was characterized using GC-MS. Hydroquinone was found in a large amount of *J. filicifolia* leaf extracts. The hydroquinone group is a subderivative of the alkaloid group, meaning it may play an important role as a growth promoter. Hydroquinone is a polyphenol compound that was used as an antioxidant. Very few simple phenols, such as hydroquinone, catechol, and orcinol, occur as free phenols in plants in low concentrations [40]. An anthraquinone (quinone derivative) found in *Cassia italic* (Pakistani tree) proved its anti-bacterial usage against pathogens such as *Corynebacterium pseudodiphthericum, Pseudomonas aeruginosa*, and *Pseudomonas pseudomalliae*. A study by Subramanian et al. [41] on fresh leaves of *Jacaranda*

https://doi.org/10.24191/sl.v18i2.27019

mimosaefolia extracted using 80% alcohol showed the presence of a hydroquinone compound. It was the first study to study the bignoniaceae family.

Polysaccharides extracted from Tamarindus indica seeds showed immunomodulatory effects that enhanced leukocyte growth and decreased cell proliferation [42]. A study by Gumgumjee et al. [43] proved that *T. indica* leaf extract were rich in polysaccharides, flavonoids, glycosides, and others. Furthermore, polysaccharides such as xylose, glucuronic acid, and galacturonic acid were found in the seed of *T. indica* [44]. Studies still need to be done on polysaccharides for *J. filicifolia*, *T. indica*, and *S. saman* extracts. This study could be the first to test polysaccharides, especially in leaves. Administration of fructose, mannose, and galactose oligosaccharides has improved growth performance, immune responses, and resistance to disease against various pathogens in different species of cultured fish. Uniquely, galactose oligosaccharides have shown responses to the mucosal and humoral immune responses in fish. In this study, the HPLC results of *J. filicifolia* indicated the presence of sucrose, galactose, glucose, and fructose oligosaccharides, suggesting that the diet supplemented with *J. filicifolia* could have positively induced fish immunity against bacterial infection. Hu et al. [45] reported that FOS supplementation in shrimp diets with partially replaced fish meals improves growth performance, immunity, and intestinal microbiota diversity. In another report, Hunt et al. [46] demonstrated that feeding rainbow trout fish with FOS reduces fish stress by increasing the total protein content. Therefore, the active compounds in plant extracts promote growth, trigger the immune system, act as antistress, have anti-bacterial and anti-parasitic properties, and stimulate appetite in fish aquaculture [47]. Consequently, these plant extracts have a great opportunity in fish culture to act as an immunostimulant against various pathogens; it is cheap to produce. The plant extracts are also easily biodegradable and environmentally friendly. Plant extracts are administered in fish aquaculture through diet, injection, or oral. The different concentrations of immunostimulants administered by diet, oral, or injection have improved the immune response in marine and freshwater fish cultures against bacterial, viral, parasite, and fungal infections [48].

CONCLUSION

It is important to treat aquatic diseases and prevent losses in fish businesses. Plant extracts have shown promising data in controlling pathogenic bacteria-borne diseases in the aquaculture industry. In this study, *J. filicifolia* has demonstrated anti-bacterial properties against all pathogens tested. Meanwhile, *J. filicifolia* showed a very potent concentration for inhibiting pathogenic bacteria. This article might be the first research to explore these plant extracts on the selected fish pathogens. However, further investigation is required to explore the potential of *J. filicifolia* leaf extract as an antimicrobial agent to combat fish diseases in the aquaculture industry.

ACKNOWLEDGEMENTS/FUNDING

The authors would like to acknowledge the School of Biological Sciences of the Universiti Sains Malaysia, USM, for using laboratory equipment and services to complete the study.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

https://doi.org/10.24191/sl.v18i2.27019

AUTHORS' CONTRIBUTIONS

All authors contributed to the study equally.

REFERENCES

[1] Alazab, A., Sadat, A., & Younis, G. (2022). Prevalence, antimicrobial susceptibility, and genotyping of Streptococcus agalactiae in Tilapia fish (Oreochromis niloticus) in Egypt. *Journal of Advanced Veterinary and Animal Research*, *9*(1), 95.

[2] Pulpipat, T., Boonyawiwat, V., Moonjit, P., Sanguankiat, A., Phatthanakunanan, S., Jala, S., & Surachetpong, W. (2023). Streptococcus agalactiae Serotype VII, an Emerging Pathogen Affecting Snakeskin Gourami (Trichogaster pectoralis) in Intensive Farming. *Transboundary and Emerging Diseases*, *2023*.

[3] Kyule, D. N., Maingi, J. M., Njeru, E. M., & Nyamache, A. K. (2022). Molecular Characterization and Diversity of Bacteria Isolated from Fish and Fish Products Retailed in Kenyan Markets. *Int J Food Sci*, *2022*.zho

[4] Zhao, Y., Yang, Q. E., Zhou, X., Wang, F.-H., Muurinen, J., Virta, M. P., Brandt, K. K., & Zhu, Y.-G. (2021). Antibiotic resistome in the livestock and aquaculture industries: Status and solutions. *Crit. Rev. Environ. Sci. Technol.*, *51*(19), 2159-2196.

[5] Kuebutornye, F. K., & Abarike, E. D. (2020). The contribution of medicinal plants to tilapia aquaculture: a review. *Aquaculture international*, *28*(3), 965-983.

[6] Tadese, D. A., Song, C., Sun, C., Liu, B., Liu, B., Zhou, Q., Xu, P., Ge, X., Liu, M., & Xu, X. (2022). The role of currently used medicinal plants in aquaculture and their action mechanisms: A review. *Reviews in Aquaculture*, *14*(2), 816-847.

[7] Abidin, Z. U., Hassan, H. U., Masood, Z., Rafique, N., Paray, B. A., Gabol, K., Shah, M. I. A., Gulnaz, A., Ullah, A., & Zulfiqar, T. (2022). Effect of dietary supplementation of Neem, Azadirachta indica leaf extracts on enhancing the growth performance, chemical composition, and survival of rainbow trout, Oncorhynchus mykiss. *Saudi J. Biol. Sci.*, *29*(4), 3075-3081.

[8] Mondal, P., Garai, P., Chatterjee, A., & Saha, N. C. (2021). Toxicological and therapeutic effects of neem (Azadirachta indica) leaf powder in hole-in-the-head (HITH) disease of fish Anabas testudineus. *Aquaculture Research*, *52*(2), 715-723.

[9] Abarike, E. D., Jian, J., Tang, J., Cai, J., Yu, H., Lihua, C., & Jun, L. (2018). Influence of traditional Chinese medicine and Bacillus species (TCMBS) on growth, immune response and disease resistance in Nile tilapia, Oreochromis niloticus. *Aquaculture Research*, *49*(7), 2366-2375.

[10] Awad, E., & Awaad, A. (2017). Role of medicinal plants on growth performance and immune status in fish. *Fish & shellfish immunology*, *67*, 40-54.

[11] Oreopoulou, A., Tsimogiannis, D., & Oreopoulou, V. (2019). Extraction of polyphenols from aromatic and medicinal plants: an overview of the methods and the effect of extraction parameters. *Polyphenols in plants*, 243-259.

[12] Khanahmadi, M., Rezazadeh, S., & Taran, M. (2010). In vitro antimicrobial and antioxidant properties of Smyrnium cordifolium Boiss.(Umbelliferae) extract. *Asian journal of plant sciences*, *9*(2), 99-103.

[13] Sofowora, A. (1993). Recent trends in research into African medicinal plants. *J. Ethnopharmacol.*, *38*(2-3), 197-208.

https://doi.org/10.24191/sl.v18i2.27019

[14] Tiwari, B. K., Abidi, A. B., Rizvi, S. I., & Pandey, K. B. (2016). Phytochemical screening and evaluation of antioxidant potentials of some Indian medicinal plants and their composite extract. *Annals of Phytomedicine*, 5(1), 99-103

[15] Khattab, R., Gaballa, A., Zakaria, S., Ali, A. A., Sallam, I., & Temraz, T. (2012). Phytochemical Analysis of Avicennia marina and Rhizophora mucronata by GC-MS. *Catrina: The International Journal of Environmental Sciences*, *7*(1), 115-120.

[16] Murthy, K., & Kiran, B. (2013). Review on usage of medicinal plants in fish diseases. *Int J Pharm Bio Sci*, *4*, 975-986.

[17] Kamble, M. T., Gallardo, W., Yakupitiyage, A., Chavan, B. R., Rusydi, R., & Rahma, A. (2014). Antimicrobial activity of bioactive herbal extracts against Streptococcus agalactiae biotype 2. *Int. J. Basic Appl. Biol*, *2*(3), 152-155.

[18] Gwiazdowska, D., Uwineza, P. A., Frąk, S., Juś, K., Marchwińska, K., Gwiazdowski, R., & Waśkiewicz, A. (2022). Antioxidant, antimicrobial and antibiofilm properties of Glechoma hederacea extracts obtained by supercritical fluid extraction, using different extraction conditions. *Applied Sciences*, *12*(7), 3572.

[19] Gebreyohannes, G., Nyerere, A., Bii, C., & Berhe Sbhatu, D. (2019). Determination of Antimicrobial Activity of Extracts of Indigenous Wild Mushrooms against Pathogenic Organisms. *Evidence-Based Complementary and Alternative Medicine*, *2019*, 6212673. https://doi.org/10.1155/2019/6212673

[20] Olawuwo, O. S., Famuyide, I. M., & McGaw, L. J. (2022). Anti-bacterial and antibiofilm activity of selected medicinal plant leaf extracts against pathogens implicated in poultry diseases. *Front Vet Sci*, *9*, 820304.

[21] Tellería Rios, M., Sgarbieri, V., & Amaya, J. (1978). Chemical and biological evaluation of quinua (Chenopodium quinoa Willd). Effect of the extraction of saponins by heat treatment. *Archivos latinoamericanos de nutricion*, *28*(3), 253-263.

[22] Roberto, P. M., Anunciação, P. C., Della Lucia, C. M., Pinheiro, S. S., de Souza, E. C. G., & Pinheiro-Sant'Ana, H. M. (2021). Macronutrients, vitamins, minerals and bioactive compounds in fresh and dehydrated basil (Ocimum basilicum) and its hot and cold infusions. *Acta Scientiarum. Technology*, *43*, e55423-e55423.

[23] Oubaid, E., Chabuck, Z., Al-Saigh, R., Hindi, N., & Kadhum, S. (2022). Pathogenic and antimicrobial properties of aquatic extracts of Viscus album.

[24] Mostafa, N. M., Eldahshan, O. A., & Singab, A. N. B. (2014). The genus Jacaranda (Bignoniaceae): an updated review. *Pharmacognosy Communications*, *4*(3), 31-39.

[25] Arulpriya, P., Lalitha, P., & Hemalatha, S. (2010). Antimicrobial testing of the extracts of Samanea saman (Jacq.) Merr. *Der pharma chemica*, *2*(6), 78-83.

[26] Adeniyi, O. V., Olaifa, F. E., Emikpe, B., & Ogunbanwo, S. (2017). Phytochemical components and anti-bacterial activity of Tamarindus indica Linn. extracts against some pathogens. *Environment*, *7*, 14.

[27] Sam Hagan, M. A., Omojola, A. B., & Donkoh, A. (2019). Assessment of Samanea saman Whole Pod Extract as an Antimicrobial Agent and its Effect on Chicken Patties.

[28] Li, A.-N., Li, S., Zhang, Y.-J., Xu, X.-R., Chen, Y.-M., & Li, H.-B. (2014). Resources and biological activities of natural polyphenols. *Nutrients*, *6*(12), 6020-6047.

[29] Prasad, R. N., Viswanathan, S., Devi, J. R., Nayak, V., Swetha, V., Archana, B., Parathasarathy, N., & Rajkumar, J. (2008). Preliminary phytochemical screening and antimicrobial activity of Samanea saman. *Journal of Medicinal Plants Research*, *2*(10), 268-270.

[30] Faddeeva, M., & Beliaeva, T. (1997). Sanguinarine and ellipticine cytotoxic alkaloids isolated from well-known antitumor plants. Intracellular targets of their action. *Tsitologiia*, *39*(2-3), 181-208.

https://doi.org/10.24191/sl.v18i2.27019

[31] Alabri, T. H. A., Al Musalami, A. H. S., Hossain, M. A., Weli, A. M., & Al-Riyami, Q. (2014). Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of Datura metel L. *Journal of King Saud University-Science*, *26*(3), 237-243.

[32] Chakraborty, S. B., & Hancz, C. (2011). Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture*, *3*(3), 103-119.

[33] Javed, B., Nawaz, K., & Munazir, M. (2020). Phytochemical analysis and anti-bacterial activity of tannins extracted from Salix alba L. against different gram-positive and gram-negative bacterial strains. *Iranian Journal of Science and Technology, Transactions A: Science*, *44*(5), 1303-1314.

[34] Parekh, J., & Chanda, S. (2008). Evaluation of antimicrobial activity of Terminalia chebula Retz. Fruit in different solvents. *Journal of herbs, spices & medicinal plants*, *13*(2), 107-116.

[35] Dindamo, L., Jagathala Mahalingam, S., Getachew, A., & Kebede, A. (2022). Anti-bacterial Activities of Ethanol Extracts from Croton Macrostachyus (Hochst. Ex Delile) and Justicia Schimperiana (Hochst. Ex Nees) Bark and Leaf against certain Pathogenic Bacteria.

[36] Koczurkiewicz, P., Klaś, K., Grabowska, K., Piska, K., Rogowska, K., Wójcik-Pszczoła, K., Podolak, I., Galanty, A., Michalik, M., & Pękala, E. (2019). Saponins as chemosensitizing substances that improve effectiveness and selectivity of anticancer drug—Minireview of in vitro studies. *Phytotherapy Research*, *33*(9), 2141-2151.

[37] Cruz, M. d. F. S. J., & Pereira, G. M. (2023). Structure-activity relationship of triterpenoid saponins: Biological properties and commercial applicabilities. *Revista Fitos*, *17*(2), 295-316.

[38] Dos Santos, L. R., Alía, A., Martin, I., Gottardo, F. M., Rodrigues, L. B., Borges, K. A., Furian, T. Q., & Córdoba, J. J. (2022). Antimicrobial activity of essential oils and natural plant extracts against Listeria monocytogenes in a dry-cured ham-based model. *Journal of the Science of Food and Agriculture*, *102*(4), 1729-1735.

[39] Mando, Z., Al Zarzour, R. H., Alshehade, S., Afzan, A., Shaari, K., Hassan, Z., Mahror, N., & Zakaria, F. (2024). Terpenoids and Triterpenoid Saponins: Future Treatment for Depression. *Current Traditional Medicine*, *10*(2), 51-68.

[40] Jiang, Q., Wang, S., Yang, Y., Luo, J., Yang, R., & Li, W. (2023). Profiles of Free and Bound Phenolics and Their Antioxidant Capacity in Rice Bean (Vigna umbellata). *Foods*, 12.

[41] Subramanian, S., Nair, A., Rodriguez, E., & Mabry, T. J. (1972). Polyphenols of the leaves of Majorana hortensis. *Current Science*, 202-204.

[42] Kuru, P. (2014). Tamarindus indica and its health related effects. *Asian Pacific Journal of Tropical Biomedicine*, *4*(9), 676-681.

[43] Gumgumjee, N. M., Khedr, A., & Hajar, A. (2012). Antimicrobial activities and chemical properties of Tamarindus indica L. leaves extract. *Afric J Microb Res*, *6*(32), 6172-6181.

[44] Ammar, N. M., Singab, A. N. B., El Ahmady, S. H., El Ansarry, A., Haggag, E. G., & Shabban, R. S. (2010). Phytochemical and Biological Studies of Some Polysaccharides Isolated From Aloe, Tamarindus, Opuntia, and Citrus. *JASMR*, *5*(2), 141-152.

[45] Hu, X., Yang, H. L., Yan, Y. Y., Zhang, C. X., Ye, J. d., Lu, K. L., Hu, L. H., Zhang, J. J., Ruan, L., & Sun, Y. Z. (2019). Effects of fructooligosaccharide on growth, immunity and intestinal microbiota of shrimp (Litopenaeus vannamei) fed diets with fish meal partially replaced by soybean meal. *Aquaculture Nutrition*, *25*(1), 194-204.

[46] Hunt, A. Ö., Çetinkaya, M., Yılmaz, F. Ö., Yıldırım, M., Berkoz, M., & Yalın, S. (2019). Effect of dietary supplementation of inulin on growth performance, digestion enzyme activities and antioxidant status of rainbow trout (Oncorhynchus mykiss). *Turkish Journal of Agriculture-Food Science and Technology*, *7*(9), 1344-1353.

https://doi.org/10.24191/sl.v18i2.27019

[47] Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture, 433*, 50-61.

[48] Vijayaram, S., Sun, Y.-Z., Zuorro, A., Ghafarifarsani, H., Van Doan, H., & Hoseinifar, S. H. (2022). Bioactive immunostimulants as health-promoting feed additives in aquaculture: A review. *Fish & Shellfish Immunology*.

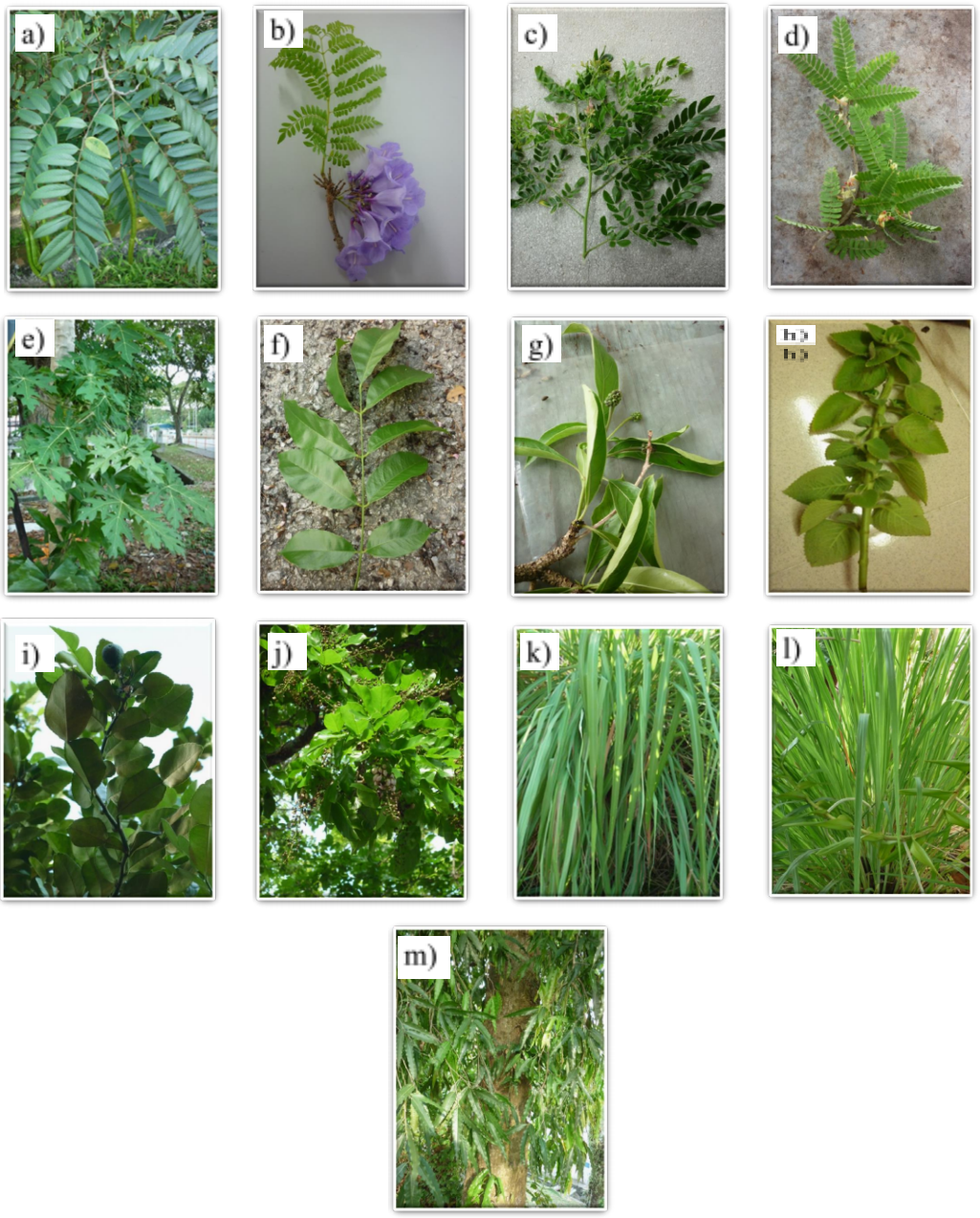
SUPPLEMENTARY FILES

Supplementary S1. The photograph of 13 plants used in the study. a) Senna spectabilis local name kasia kuning; b) Jacaranda filicifolia, local name Jambul merak; c) Samanae saman, local name Pukul Lima; d) Tamarindus indica, local name Asam Jawa; e) Carica papaya, local name Betik; f) Andira inermis, local name Brown heart; g) Morinda elliptica, local name Mengkudu kecil; h) Coleus aromaticus benth, local name Lemuju; i) Citrus hystrix, local name Limau purut; j) Milletia pinnata, local name Mempari; k) Cymbopogon nardus, local name Serai Wangi; l) Cymbopogon citratus, local name Serai; m) Polyalthia longifolia, local name Asoka.

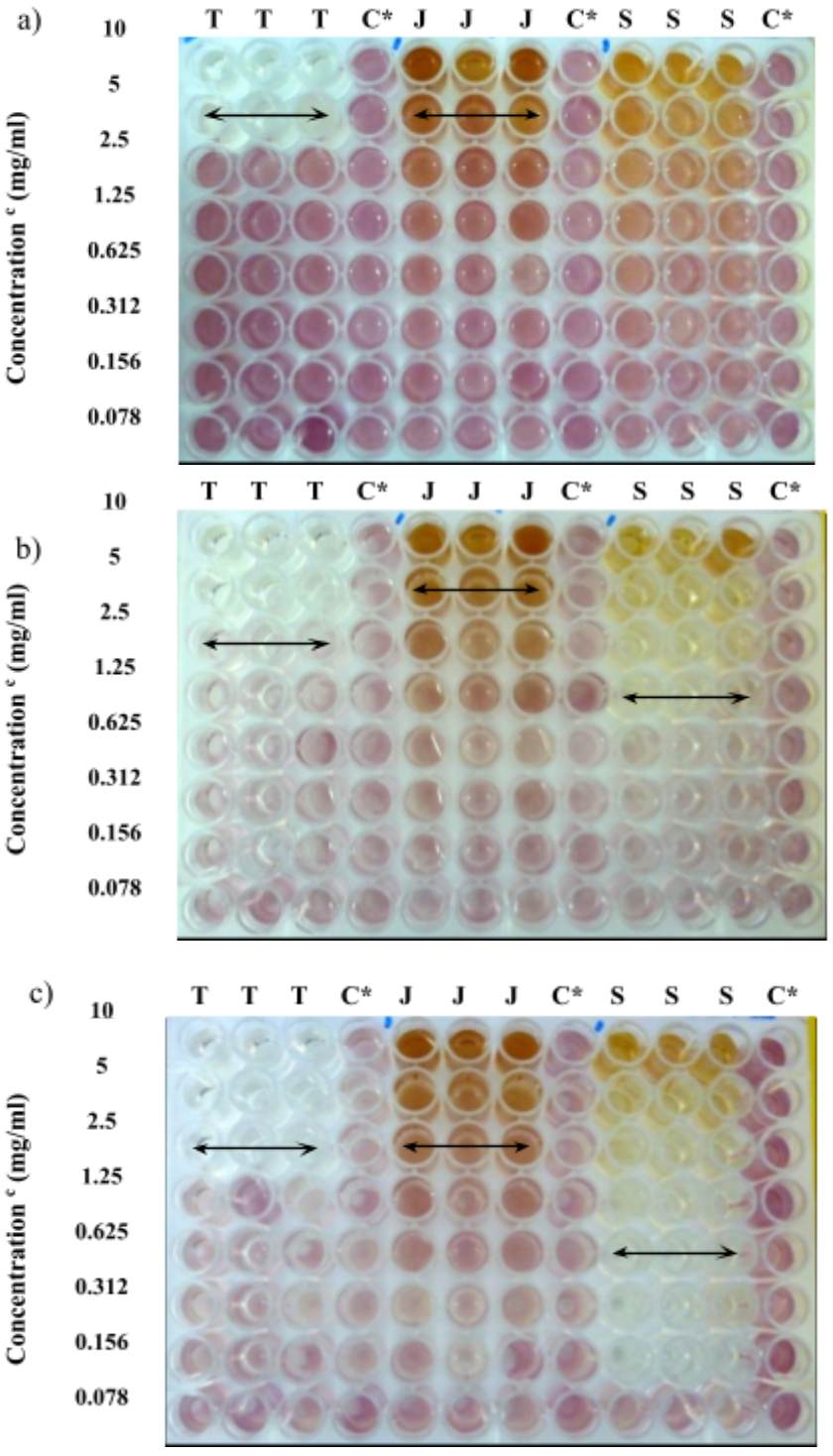
Supplementary S2. Photograph of microplate from minimum inhibitory concentration (MIC) test of Tamarindus indica (T), Jacaranda filicifolia (J) and Samanae saman (S) against a) A. hydophilla. b) S. agalactiae and c) S. xylosus. C* denotes a control.

Supplementary S3. Paragraph of the results of different phytochemical tests. a) alkaloids test, b) saponins test, c) flavonoids test, d) tannins with Polyphenolic and e) triterpenes or Steroids test on the selected plants. Note: J- Jacaranda filicifolia, T- Tamarindus indica, S- Samanae saman
[1]

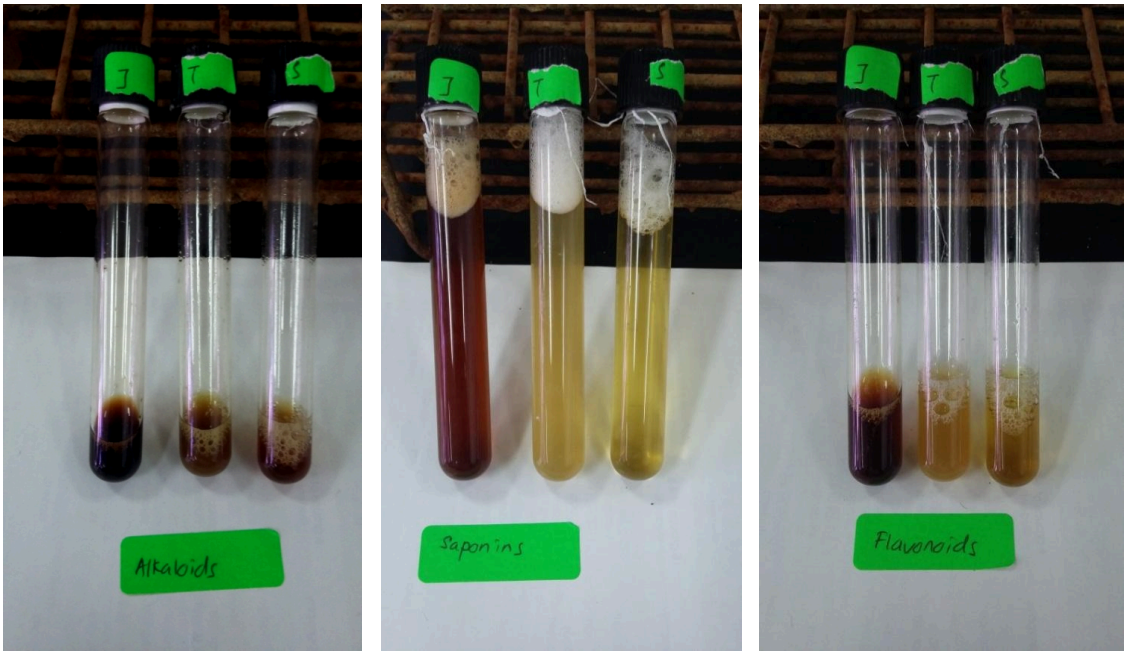
https://doi.org/10.24191/sl.v18i2.27019

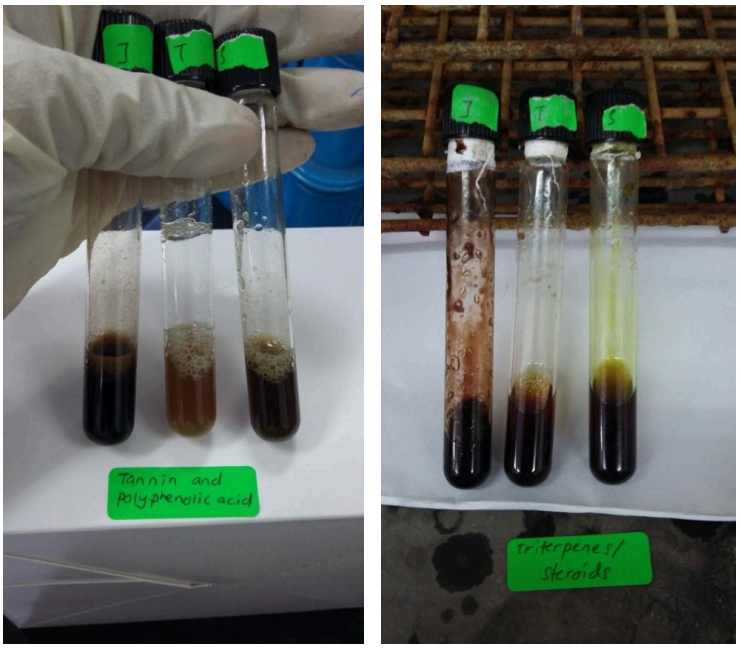


Supplementary S1



Supplementary S2





Supplementary S3