Phytochemical Screening and Antimicrobial Activity of Roselle (*Hibiscus sabdariffa* L.) Flower Extract Against *Aeromonas hydrophila*

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Abstract

Medicinal plants as an antimicrobial agent may provide an alternative way to replace the use of antibiotics to control disease agents in aquaculture. Roselle flowers (*Hibiscus sabdariffa* L.) has been used in many sectors as a source of functional food, natural coloring agents as well as antimicrobial agents. The objectives of this study were to evaluate the phytochemical compound in methanolic extract of roselle flower and their antimicrobial activity against *Aeromonas hydrophila*. The phytochemical composition of roselle flower was evaluated using phytochemical screening and FTIR. While the antimicrobial activity was performed by using the disc diffusion agar and co-culture with *A. hydrophila*. The results of phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, steroids, triterpenoids, and tannins. The results of FTIR revealed that Roselle flower extract had the main phenolic compounds. The result of disc diffusion and co-culture method indicated that the roselle flower extract had antibacterial activity against *A. hydrophila*. This antibacterial activity depended on the concentration applied.

Keywords: antibiotics, Co-culture, Disk diffusion, flavonoid, *Hibiscus* sp.

INTRODUCTION

Aeromonas hydrophila is a common bacteria in freshwater habitats throughout the world [1]. It cause diseases both in human and animals including fish and shrimp [2]. Bacterial infections with *Aeromonas hydrophila* as a disease agent have resulted in heavy losses up to 80% and economic loss to fish farmers [3,4].

Usually, many farmers have used antibiotics to control this disease. However, antibiotics have become the major factor for the emergence and dissemination of multi-drug-resistant strains of several groups of microorganisms [5]. An effort to overcome the negative effects of antibiotic application is the use of medicinal plants. Plants are rich in a wide variety of secondary metabolites such as *tannins, alkaloids*, and *flavonoids*, which have been found in vitro to have antimicrobial properties [6]. There have been several reports on the antimicrobial activity of different herbal extracts [7–9].

Hibiscus sabdariffa L., well-known as roselle, is a common flower plant grown worldwide. More than 300 species of hibiscus can be found around the world [10]. Roselle have been used in many sectors as a source of functional food, natural coloring and antimicrobial agents [11–13]. Several studies found that roselle flower have significant antimicrobial activities against several strain of bacteria such as *Micrococcus luteus, Serratia mascences, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella enterica*, *Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Escherichia coli,* and *Bacillus cereus* [14,15]. Yet, no studies found to reveal the antimicrobial activities against *A. hydrophila* as a bacterial disease agent in aquaculture. Therefore, this study was intended to evaluate the phytochemical screening and antimicrobial activity of Roselle flower extract on *A. hydrophila* indigenous aquatic.

MATERIAL AND METHODS

Preparation of Roselle flower Extract

Roselle flower (*H. sabdariffa* L.) was originated from house of medicinal plant, Batu, East Java. The flowers were air-dried and grounded into fine powder using an electric blender. Extraction process was performed based on the methods of [16] with some modification. A hundred (100) g of air-dried powder was mixed with 500 ml of methanol solvent and then was kept for 24h. Later, it filtered through Whatman filter paper (no. 42) and centrifuged at 5.000 g for 10 min. The extract was evaporated using a rotary evaporator at 40°C.

Phytochemical Composition and FTIR of Roselle Flower Extract

Phytochemical screening was carried out to confirm the presence of alkaloids, flavonoids, saponins, triterpenoids, steroids, and tannins using the standard method [17]. FTIR analysis was also conducted to elucidate the

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phytochemical composition of roselle flower extract based on standard methods [18]. Roselle flower extract (1 mg) was homogenously mixed with 100 mg KBr and pressed in to pellet. FTIR spectra were recorded in the range 4000-400 cm-1 in FTIR spectroscopy (OPUS 4.2, Karlsruhe, Germany).

Bacterial preparation

Isolate of *Aeromonas hydrophila* was originated from Jepara Brackishwater Aquaculture Center. This bacterium was kept in *Trypticase Soy Agar* (TSA) media at 4°C and sub-cultured *Trypticase Soy Broth* (TSB) in overnight before use.

Antimicrobial assay

Concentration of Roselle flower extract used in this study were 0, 1, 10, 100, 500 and 1,000 $mg.ml^{-1}$ which diluted in 10% DMSO. Antimicrobial assay was carried out using modified disc diffusion method and co-culture method. The modified disc diffusion method was performed based on [19]. The overnight bacterial suspension was adjusted to the concentration of 10^7 $CFU.mL^{-1}$ and seeded (0.1 mL) on Muller Hinton Agar (MHA) plate. Each treatment (5 µL) of Roselle flower extract was applied in to MHA plate. A positive control antibiotic (Chloramphenicol 5 $mg.mL^{-1}$) and a negative control (without extract) was used in this assay. MHA plates were incubated at 37°C for 24 hours. Antimicrobial activities was evaluated by measuring the inhibition zone. The co-culture method was performed based on [20]. In 100 mL TSB, *A. hydropila* (1 mL) was inoculated with an initial bacterial count of 10^7 $CFU.mL^{-1}$. The flower extract was added in to the flask at the varied concentration above. Those co-culture flasks were incubated at 37°C on shaker (120 rpm) for 24 hours. The *A. hydrophila* was enumerated by using the standard plate method. All treatments were performed in triplicates.

RESULT AND DISCUSSION

Phytochemical Composition

The phytochemical analysis was performed to confirm the presence of alkaloids, flavonoids, saponins, steroids, triterpenoids and tannins in the extract of roselle flower. The results of phytochemical analysis were given in the Table 1. The results showed that all the compound tested were found in roselle flower extracted with methanol solvents. Results of this phytochemical analysis was also supported by the earlier studies [21]. They reported that the flower of roselle contained group of alkaloids, flavonoids, saponins, steroids, triterpenoids and tannins. Other plants that have antimicrobial activities were also had these compounds [22–24].

Table 1. The Phytochemical Analysis of Roselle Flower Extract

Metabolites	Results
Alkaloids (Dragendroff)	+
Alkaloids (Mayer)	++
Flavonoids	+++
Saponins	+
Steroids	+
Triterpenoids	+
Tannins	+

Notes: +++ = strong, ++ = medium, + = weak intensity [25]

FTIR Analysis Results

The spectrum of FTIR was designed to identify the functional group of bioactive compound. This assignment was confirmed based on the peaks value in the IR radioation region. The FT-IR spectrum of roselle flower extract is presented in Fig. 1, while the spectra is interpreted in Table 2. The results indicated that there were various functional groups present in the roselle flower extract.

Table 2. Assignment of FT-IR Absorption Bands in the Roselle Flower Extract

Absorption frequency (cm^{-1})	Bond	Tentative assignment
3,419.71	O-H	Alcohol, phenol
2 928.91	C-H	Alkanes
1 743.03	C = O	Aldehydes
1,630.18	C = C	Alkenes
1,384.12	C-H aliphatic	Alkanes
1,228.37	C-O	Carboxylic acid
1,065.59	C-O	Carboxylic acid
523.34	C-H	Aromatics

The result of FTIR analysis confirmed the presence of phenol, alkanes, aldehydes, carboxylic acid, and aromatics. Allegation that the results of the isolate are phenolic compounds where the benzene group binds to one OH group with a wide and sharp inclination with absorption at the wave number area 3419.71 cm^{-1} and 1384.12 cm^{-1}. And reinforced functional groups OH, C = C, C = O, C-H aromatic [26–28]. Based on the results of phytochemical screening analysis and FTIR analysis, it can be linked that flavonoids and positive tannins contained in roselle flower extract are derivatives of phenol [29,30]. According to previous study [31], phenol derivatives contained in plants have antimicrobial activity.

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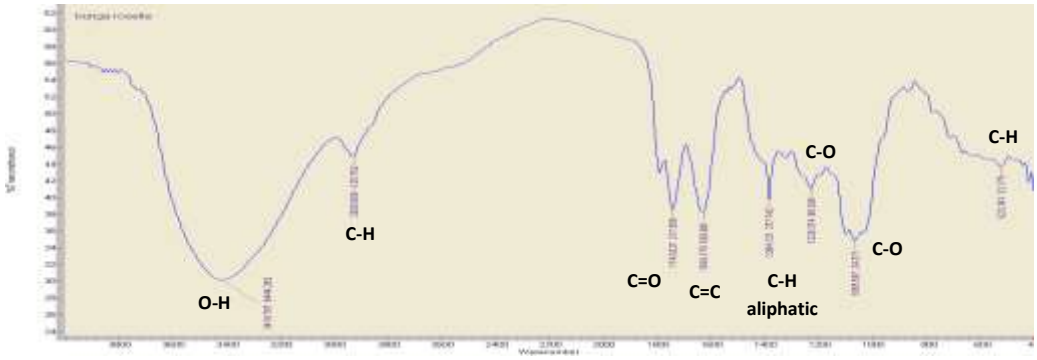

Figure 1. FTIR Test Results of Rosella Flower Extract (H. sabdariffa L.).

Antimicrobial Assay

The results of antimicrobial assay of *H. sabdariffa* L. provide valuable information and highlight the potential value of this plant in aquaculture drug development. Classification was made based on the width of the clear zone formed. The results of the antimicrobial assay of the methanolic extract of *H. sabdariffa* L. against *A. hydrophila* can be seen in Table 3.

Table 3. Antimicrobial Testing of Roselle Flower Extract against *A. hydrophila* by Using Disc Diffusion

Concentration of *Roselle flower extract* (mg.L^{-1})	Clear Zone Diameter (mm)	Inhibitory Responses
Control (-)	0.00±0.0^{a}	Weak
1	0.00±0.0^{a}	Weak
10	2.19±0.0^{b}	Weak
100	7.12 ±0.8^{c}	Medium
500	15.02±0.5^{d}	Strong
1000	18.37±0.3^{e}	Strong
Control (+)	21.47±0.7^{f}	Strong

Description: ≤5mm =weak, 5-10 mm= medium,10-20 mm= strong [32]

Table 4. Co-Culture Test Result

No	Concentration of *Roselle flower extract* (mg.L^{-1})	Bacterial count (CFU. mL^{-1})
1	Control (-)	2.70 E18
2	1	2.66 E18
3	10	1.85 E14
4	100	1.08 E11
5	500	8.6. E09
6	1000	5.3. E08
7	Control (+)	0

The result of disc diffusion and co-culture method indicated that the roselle flower extract had an antimicrobial activity against *A. hydrophila* (Table 4). Some studies also revealed the antibacterial activities of Roselle extract against some bacteria such as *Streptococcus mutans* [33], *Campylobacter jejuni, C. coli, C. fetus* [34]. The antibacterial activities detected in this study were concentration-dependent. The higher the concentration of Roselle flower extract, the higher the antibacterial detected against *A. hydrophila*. It can be seen from Table 3 that the concentration of roselle flower extract which categorized withs strong ability to inhibit the growth of *A. hydrophila* was started from 500 mg.L^{-1}. In line with that finding, the results of co-culture assay showed that the significant reduction of *A. hydrophila* count was also from the same concentration. The antimicrobial activity was influenced by several factors such as the concentration of extract, the content of antibacterial compounds, the diffusion power of extracts and the type of bacteria [35].

The ability of Roselle flower extract to inhibit the growth of *A. hydrophila* depend on their bioactive compound. The main compound of Roselle are organic acids, anthocyanins, polysaccarides and flavonoids [36]. Based on the results of the phytochemical screening test and FTIR analysis in this study, it was found that Roselle flower extract had the main phenolic compounds such as flavonoids, tannins. These phenolic compounds have the ability to form certain complex structures on bacterial cell walls. Furthermore, with the number of hydroxyl groups present in the phenolic ring there will be an increase in hydroxylation, and with increased hydroxylation, antimicrobial activity will increase [37].

Other compounds which were also found in Roselle methanol extract are alkaloids, saponins, steroids, and triterpenoids. Those compounds have also antimicrobial activities by damaging the structure of cell walls and changing the permeability of cell cytoplasmic membranes [38], [39]. Changes and damage to the cytoplasmic membrane cause leakage of intracellular material and cell metabolic disorders [40].

CONCLUSION

The phytochemical analysis of methanol extract roselle flowers confirmed the presence of

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alkaloids, saponins, steroids, triterpenoids, tannins and strong intensity of flavonoids. The results of FTIR revealed that Roselle flower extract had the main phenolic compounds. The antimicrobial assay found that the roselle flower extract had antibacterial activities against *A. hydrophila*. The antibacterial activities depended on the concentration of Roselle flower extract.

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