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# Antibacterial activity of *Nypa fruticans* leaf extract to *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Escherichia coli*

## *Actividad antibacteriana del extracto de hoja de* Nypa fruticans *frente a* Aeromonas hydrophila*, Pseudomonas aeruginosa y* Escherichia coli

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**ABSTRACT**

This study was carried out to analyze the group of secondary metabolites of leaf extract of *Nypa fruticans* which can be bioactive compounds to pathogenic bacteria *Aeromonas hydrophila, Pseudomonas aeruginosa,* and *Escherichia coli*. It was carried out from March to April 2021, and samples were collected from Mengkapan Village, Sungai Apit District, Siak Regency, Riau Province, Indonesia. The method used in this study was experimental, using a completely randomized design (CRD) with 1 factor (leaf extract concentration) with 4 treatment levels, namely 12.5%, 25%, 50%, and 100%, paired with negative and positive control with 3 replications. The bioactive substances were extracted by using ethanol solvent. The effectiveness of antibacterial substances was measured using the paper disc diffusion agar method. The phytochemical analysis released that leaf extract contained alkaloids, phenolics, flavonoids, tannins, terpenoids. The extract inhibited the growth of pathogenic bacteria *A. hydrophila*, and *P. aeruginosa* very strongly (inhibition zone >20 mm), with inhibition diameters ranging from18.56-26.16 mm and 21.00-28.10 mm, respectively. Meanwhile, for *E. coli*, the inhibitory zone was weak (< 5 mm), in which the diameter of inhibition ranged from 2.80-6.46 mm.

**Keywords:** bioactive compounds, mangrove extract, antibacterial, flavonoids, alkaloids.

***RESUMEN***

*El objetivo del estudio fue analizar el grupo de metabolitos secundarios del extracto de hoja de Nypa fruticans que pueden ser compuestos bioactivos para las bacterias patógenas* Aeromonas hydrophila, Pseudomonas aeruginosa *y* Escherichia coli*. El ensayo se llevó a cabo desde marzo a abril de 2021 y se recolectaron muestras en la aldea de Mengkapan, distrito de Sungai Apit, regencia de Siak, provincia de Riau, Indonesia. El diseño experimental fue de bloques completamente al azar con un factor (concentración de extracto de hoja) y cuatro tratamientos: 12,5%; 25%; 50% y 100%, emparejados con un control negativo y positivo con, tres repeticiones. Las sustancias bioactivas se extrajeron usando etanol como solvente. La eficacia de las sustancias antibacterianas se midió utilizando el método agar de difusión en disco de papel. El análisis fitoquímico reveló que el extracto de hoja contenía alcaloides, fenoles, flavonoides, taninos, terpenoides. El extracto inhibió muy fuertemente el crecimiento de las bacterias patógenas* A. hydrophila *y* P. aeruginosa *(zona de inhibición >20 mm) con un diámetro de inhibición que osciló entre 18,56 y 26,16 mm y entre 21,00 y 28,10 mm, respectivamente. Para* E. coli*, la zona de inhibición fue débil (< 5 mm), en la que el diámetro de inhibición osciló entre 2,80 y 6,46 mm.*

***Palabras clave:*** *compuestos bioactivos, extracto de nipa, antibacteriano, flavonoides, alcaloides.*

## Introduction

*Nypa fruticans* is a species of palm plant from 35 genera of palms in Indonesia. This plant grows a lot in tidal ecosystems and is grouped into a mangrove forest community. Various species of mangrove plants contain secondary metabolites, such as alkaloids, flavonoids, steroids, terpenoids, saponins, and others. This metabolite is a bioactive substance that some plants can be used as medicinal ingredients. The functions of secondary metabolites are as a body defense for

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plants from pests and disease-causing pathogens and as growth-regulating hormones (Ebana *et al*., 2015; Gazali *et al*., 2019; Prasad *et al*., 2013; Yusof *et al*., 2019).

*Aeromonas hydrophila* is Gram-negative, short rod-shaped, aerobic and facultative anaerobic, non-sporing, motile with one flagellum, living at a temperature range of 25-30ºC and can cause symptoms of septicemia hemorrhagic disease to fish and gastroenteritis, diarrhea and extraintestinal in humans. *Pseudomonas aeruginosa* is a Gram-negative bacterium, single cells, straight or curved rods, but not helical in shape. Typically, size 0.5 -1.0 µm in motile and polar flagella, monotric or multitricus, and opportunistic pathogenic bacteria produce several exotoxins and other bioactive compounds. *Escherichia coli* is rod-shaped with a size of 1.1 - 1.5 µm - 2.0 - 6.0 µm, has a capsule-like shape. These bacteria can live and thrive at a temperature of 37 ºC with a pH of 4.4-9 and may cause diarrhea through water and food or direct contact with animals and people (Batra *et al*., 2015; Strateva and Yordanov. 2009; Syawal *et al*., 2020). This study aimed to carry out a phytochemical analysis and examine the antibacterial activity of *Nypa fruticans* mangrove leaf extract against the pathogenic bacteria *A. hydrophila, E. coli,* and *P. aeruginosa*.

## Materials and methods

### Place, time and design

This research was carried out from March to April 2021. Samples of *N. fruticans* leaves were collected from the mangrove forest area of Mengapan Village, Sungai Apit District, Siak, Riau, Indonesia. The method used in this study is experimental, using a Completely Randomized Design (CRD) with 1 factor from 4 treatment levels, namely 12.5%, 25%, 50%, and 100%, negative control and positive control and with 3 times replications.

### Preparation of mangrove leaf extract

The fresh leaf samples were cleaned using clean water, air-dried, and then sliced thinly. The leaf slices were dried and blended until smooth and soaked (maceration) with ethanol solution (96%) for 24 hours. The solution was filtered with filter paper; the remainder of the first filter was macerated in the same way, until it produced a clear colored filter. The filtrate was collected and evaporated using a rotary vacuum evaporator to separate the solvent at a temperature of 60ºC until the solvent had evaporated, and a crude extract of *N. fruticans* mangrove leaves was obtained.

### Phytochemical analysis

Saponin detection. A total of 0.1 g of sample was put into a beaker, then added to 10 ml of hot water and boiled for 5 minutes. The mixture was filtered, and the filtrate was used as a test solution. The filtrate was put into a closed test tube, then shaken for ± 10 seconds and left for 10 minutes, added 1 ml of 2 M HCL. The formation of a stable foam indicated the presence of saponins.

Detection of phenols and tannins. Mangrove leaf filtrate was added with 3 drops of FeCl3; the presence of phenolic compounds was indicated by a dark green or blue color.

Flavonoid detection. Mangrove leaf extract filtrate was added with 5 drops of concentrated Mg and HCl and shaken vigorously until a layer was formed. Reddish yellow to red color indicates that the positive sample contains flavonoid compounds.

Detection of steroids and terpenoids. Firstly, the chloroform liquid was prepared, then poured into 2 holes of the drip plate and fanned to dry; then, to the 2 holes of the drip plate was added concentrated anhydrous acetic acid and concentrated $H_2SO4$, the presence of steroid compounds was shown in green and terpenoids in purple color.

Alkaloid detection. A total of 0.05 g of filtrate sample was put into a test tube and then added drops of $H_2SO4$ and shaken until completely mixed. Then it is poured into a drip plate and dripped with Meyer's reagent by seeing a white precipitate, Wagner's reagent by seeing a brown precipitate, and Dragendorff's reagent with an orange precipitate. If there is a precipitate, the sample is said to be positive for alkaloids.

### Antibacterial activity of *N. fruticans* leaf extract

Aseptically *N. fruticans* leaf extract solutions were prepared at concentrations of 12.5%, 25%, 50%, and 100%. The antibacterial activity against pathogenic bacteria (*A. hydrophila, P. aeruginosa,*

*and E. coli*) was carried out using the paper disc diffusion agar method. Bacterial isolates rejuvenated in nutrient broth were taken as much as 50 µl and spread using a glass rod on MHA media. Paper discs dripped with *N. fruticans* leaf extract with concentrations of 12.5%, 25%, 50%, and 100%, positive and negative controls were placed on the inoculated plates and incubated for 24 hours. The inhibition zone was examined by measuring the clear zone diameter around the paper disc using a caliper (Effendi *et al*., 2020).

## Results and discussion

### Phytochemical analysis of leaf extract of *N. fruticans*

In this study, the analysis was carried out to determine the content of groups of bioactive compounds present in the mangrove leaves of *N. fruticans*. The alkaloids analysis showed positive results, indicated by a change in the color of the extract to orange and the presence of a precipitate. Phenolic compounds were also detected in the extract and characterized by a change in the preparation color to dark green. This mangrove leaf extract was positive for flavonoids, indicated by a reddish-yellow change in the preparation color. The presence of tannin compounds was also detected positively; this was indicated by a change in the preparation color to green. The extract was also positive for terpenoids, characterized by a change in the preparation color to purple. However, the extract did not contain saponin and steroids (Table 1).

The leaf extract of *N. fruticans* contained bioactive secondary metabolite compounds of alkaloids, phenolics, flavonoids, tannins, and terpenoids. The results obtained in this study are not much different from those reported by some previous researchers. Some researchers from Indonesia (Gazali *et al*., 2019) extracted bioactive compounds from the leaves of *N. fruticans* using methanol, ethyl acetate, and n-hexane as solvents. It was reported that the three solvents found that the bioactive substances contained were phenolics, flavonoids, tannins, saponins, and steroids. The similar result was also reported by others (Yusoff *et al*., 2015), who mentioned that the extract of *N. fruticans* contains phenolic and flavonoid compounds, which show strong antioxidant activity.

Table 1. Results of phytochemical analysis of leaf extract of *N. fruticans*.

| Nº | Active compounds | Color | Result |
|---|---|---|---|
| 1 | Saponin | No foam was formed | – |
| 2 | Phenolic | Blackish green | + |
| 3 | Tanin | Green/dark blue | + |
| 4 | Flavonoid | Reddish yellow | + |
| 5 | Steroid | Green | – |
| 6 | Terpenoid | Purple | + |
| 7 | Alcaloid | Orange with sediment | + |

Other researchers (Edu *et al*. 2015) reported that *N. fruticans* extract contains active chemical compounds, flavonoids, tannins, phenol hydroquinone, diterpenes, steroids, and saponins. From Nigeria (Ebana *et al*. 2015), it was also noticed that nipah leaf (*N. fruticans*) samples collected in the Niger Delta of the Nigerian region did not show the presence of saponins, and tannins while the alkaloid compounds were positive. Eight phenolic compounds from the endosperm extract of *N. fruticans* using high-performance liquid chromatography were identified, namely chlorogenic acid, protocatechuic acid, and kaempferol are the main phenolic compounds (Prasad *et al*. 2013).

### Antibacterial activity of *N. fruticans* leaf extract

Antibacterial activity was used to determine whether the extract inhibited the pathogenic bacteria. Inhibition was indicated by the presence of a clear zone formed around the disc filled with leaf extract. The leaf extract of *N. fruticans* was shown to inhibit the growth of *A. hydrophilla* and *P. aeruginosa* in a strong category and a weak category for *E. coli* (Figures 1, 2, and 3). For *A. hydrophila*, the widest zone of inhibition was found at a concentration of 12.5%, with an inhibition zone diameter of 26.16 mm, and the lowest at a concentration of 50% (18.56 mm). For *P. aeruginosa,* the best results were obtained at a concentration of 50%, which resulted in an inhibition zone diameter of 28.1 mm, and the lowest at a concentration of 100%, with an inhibition zone diameter of 21 mm. As for *E. coli*, the results were obtained at a concentration of 100%, which resulted in an inhibition zone of 6.46 mm, and the lowest was obtained at a concentration of 12.5% (2.8 mm). More detailed data are presented in Table 2.

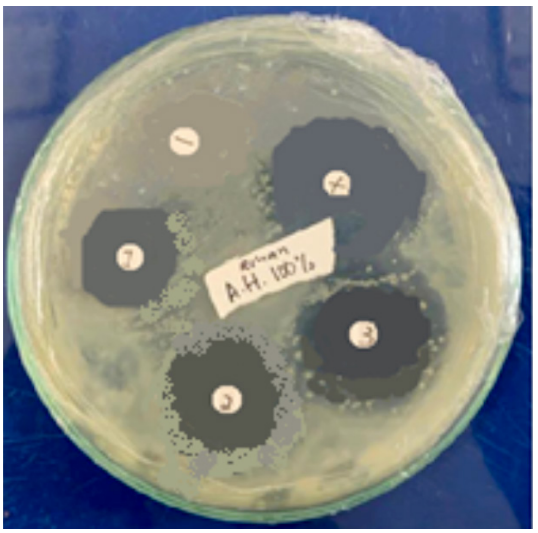


Figure 1. Inhibition zone of *N. fruticans* leaf extract against *A. hydrophila*.

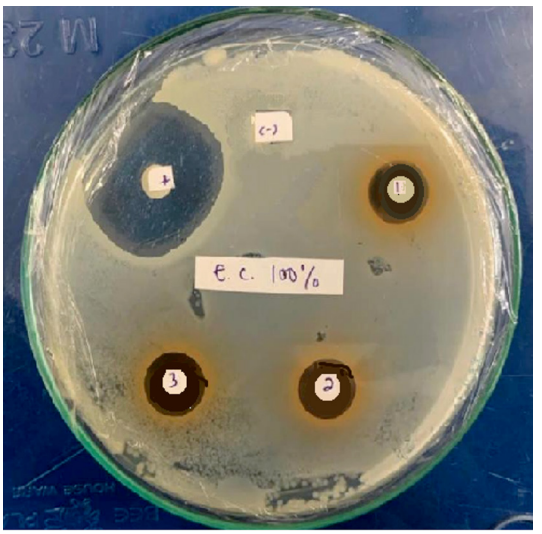


Figure 3. Inhibition zone of *N. fruticans* leaf extract against *E. coli*.

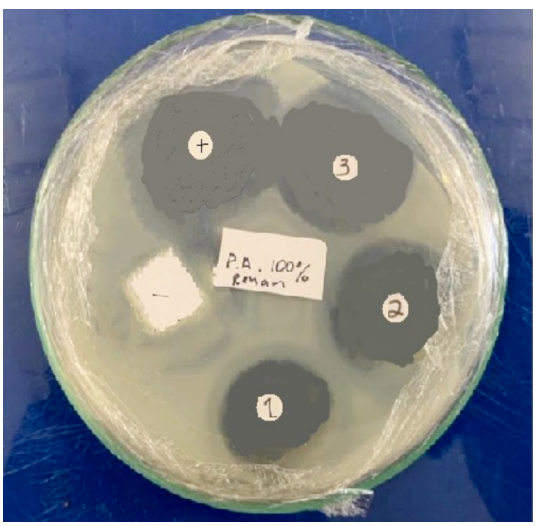


Figure 2. Inhibition zone of *N. Fruticans* leaf extract against *P. Aeruginosa*.

The extract inhibited the growth of pathogenic bacteria *A. hydrophila*, and *P. aeruginosa* very strongly (inhibition zone >20 mm); henceforth, for *E. coli*, the inhibitory zone was weak (< 5 mm). Based on the formed diameter of the inhibition zone, the antibacterial activity is classified into; weak (diameter of zone < 5 mm), moderate (diameter of the zone between 5-10 mm), strong (diameter of the zone between 10-20 mm) and classified as very strong or zone between 10-20 mm (EUCAST, 2012).

Some researchers (Cushnie *et al*. 2014; Singh *et al*. 2011) explain that the mechanism of action of antimicrobial substances such as alkaloids and flavonoids against pathogenic bacteria is thought to inhibit the work of these bacterial enzymes resulting in disruption of metabolism or the death of these bacterial cells. It can also inhibit the formation of enzymes in the form of extracellular toxins, which are virulence factors in bacteria. The mechanism of action of this antibacterial can also occur in several other ways, namely damage to cell walls, changes in cell permeability, and inhibiting protein and nucleic acid synthesis. Many factors and circumstances can affect antibacterial work. Antibacterial compounds that attack bacteria will damage their cell walls or prevent their synthesis so that it will cause the formation of cells that are sensitive to osmotic pressure or known as trauma (Trombetta *et al*., 2005; Russell *et al*., 1996; Al-Delaimy and Ali, 1970). Similar results were also reported by Rady *et al.* (2020) and Purba *et al.* (2020). These two research groups found a strong inhibitory effect of the leaf, midrib, and root extracts of *N. fruticans* to mosquito larvae in brackish water.

Tannins are substances that are widely distributed in plants, such as leaves, immature fruit, stems, and bark of mangroves. The content of tannins in immature fruit is used as an energy source. Tannins are complex phenolic compounds that can inhibit bacterial activity, so plants containing tannins are

Table 2. Antibacterial activity of leaf extract of *N. fruticans*.

| Bacteria | Leaf ectract concentration (%) | Inhibition zone (mm) | | | Positive control | Average (mm) |
|---|---|---|---|---|---|---|
| | | R-1 | R-2 | R-3 | | |
| *A. hydrophila* | 12.5 | 27.1 | 26.2 | 23.2 | 21.6 | 26.16 ± 2.62 |
| | 25 | 20.1 | 16.3 | 23.6 | 30.1 | 20.00 ± 3.65 |
| | 50 | 20.0 | 15.6 | 20.1 | 31.1 | 18.56 ± 2.56 |
| | 100 | 20.7 | 19.0 | 23.3 | 29.0 | 21.00 ± 2.16 |
| *P. aeruginosa* | 12.5 | 22.7 | 26.0 | 25.6 | 26.1 | 26.76 ± 1.68 |
| | 25 | 26.1 | 17.1 | 20.5 | 23.0 | 21.23 ± 4.54 |
| | 50 | 28.1 | 23.2 | 33.1 | 31.1 | 28.10 ± 5.00 |
| | 100 | 16.2 | 25.1 | 21.7 | 25.1 | 21.00 ± 4.49 |
| *E. coli* | 12.5 | 2.7 | 2.3 | 3.4 | 24.4 | 2.80 ± 0.5 |
| | 25 | 4.3 | 3.1 | 3.2 | 22.3 | 3.53 ± 0.6 |
| | 50 | 6.6 | 5.3 | 5.9 | 20.7 | 5.93 ± 0.3 |
| | 100 | 5.7 | 6.8 | 7.0 | 16.9 | 6.46 ± 0.4 |

R1 = Replication 1. R2 = Replication 2. R3 = Replication 3. Av = Average.

often used in the pharmaceutical field because tannins contain tannic acid, which has been used as an antiseptic (Chung *et al.* 1998; Robles 2014). Tannins can inhibit the growth and kill the fungus *Candida albicans* and inhibit the growth of bacteria (Syawal *et al.*, 2021). It is suspected that tannins can be used as an antibacterial by shrinking the cell wall or cell membrane so that it interferes with the permeability of the cell itself. Due to the disruption of permeability, the cell cannot carry out living activities, so its growth is inhibited or even dies. Tannins are polyphenols reactive with bacterial cell walls and extracellular enzymes produced by bacteria. This interaction will inhibit the transport of nutrients into cells, thereby inhibiting the growth of organisms (McSweeney *et al.* 2001).

Flavonoids are one of the most abundant natural compounds found in plant tissues. Several medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer activities (Mathesius, 2018). Inhibit many oxidation reactions, both enzymes, and non-enzymes. Flavonoids are good reducing compounds that dissolve in polar solvents, such as ethanol, methanol, butanol, acetone, dimethylsulfoxide, and water. The mechanism of action of flavonoid compounds damages cell membranes irreparably (Panche *et al.* 2016). Flavonoids are secondary metabolites of polyphenols, found widely in plants and food, and have various bioactive effects, including antiviral, anti-inflammatory, cardioprotective, antidiabetic, anticancer (Marzouk, 2016), antiaging, antioxidant (Vanessa *et al.*, 2014) and others.

Terpenoids are the largest group of secondary metabolite compounds found in many plant species and can be useful as an effective antibacterial compound in inhibiting the growth of bacteria, fungi, viruses, and protozoa. The most common mechanism of action of terpenoids consists of inhibition of the swarming motility, suppression of bacterial growth by irritating the cell wall, coagulating bacterial proteins, causing hydrolysis, and diffusion of cell fluid due to differences in osmotic pressure (Tholl 2015; Yazaki *et al.* 2017).

Alkaloid compounds have antibacterial activity. The mechanism that occurs is thought to be interfering with the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not fully formed. Disruption of peptidoglycan synthesis causes imperfect cell formation because it does not contain peptidoglycan and the cell wall only covers the cell membrane, causing cell death (Singh *et al.* 2011).

## Conclusions

The leaf extract of *N. fruticans* contained bioactive secondary metabolite compounds of alkaloids, phenolics, flavonoids, tannins, and terpenoids. The extract inhibited the growth of pathogenic bacteria *A. hydrophila*, and *P. aeruginosa* very strongly (inhibition zone ≥20 mm) with inhibition diameter ranged of 18.56-26.16 mm and 21.00-28.10 mm, respectively. Meanwhile, for *E. coli*, the inhibitory zone was weak (< 5 mm), namely the diameter ranged 2.80-6.46 mm.

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