Antioxidant Activity of Nipah Endophytic Fungi (*Nypha fruticans* Wurmb) from Tanjung Jabung Timur Jambi

Fitratul Aini[1*], Hasnaul Maritsa[1], Hesti Riany[1]

[1] Biology Study Program, Faculty of Science and Technology, Jambi University, Jambi Kampus Pinang Masak, Jln. Raya Jambi-Muaro Bulian KM.15 Mendalo Darat-36361

**email: fitratulaini47@gmail.com*

Article Info

Key word:
Mangrove
Nypa fruticans Wurmb
Endophytes
Antioxidant
DPPH

Article history:
Received: 01/02/2019
Revised: 19/06/2019
Accepted: 28/11/2019

ABSTRACT

Nypa fruticans Wurmb is a member of the Palmae tribe that lives in the Mangrove ecosystem and is known to have rich bioactive sources, such as antioxidants. The ability of N. fruticans as an antioxidant may also be owned by endophytes associated with N. fruticans. This study aims to obtain endophytes that live in the tissue of the leaves of N. fruticans and find out its ability as an antioxidant agent using synthetic free radicals 2,2 diphenil-1-picrylhydrazyl (DPPH). Samples were taken at Nipah Panjang Tanjung Jabung Timur, isolation and production were carried out at the Engineering and Biotechnology Laboratory of the Faculty of Science and Technology the University of Jambi, absorbance measurements using UV-VIS at λ 517 nm were carried out at CCRC Jambi University. The antioxidant activity of isolates is shown from the inhibitory concentration (IC50) which causes the loss of 50% of DPPH free radicals. From the results obtained that the six isolates (DN01, DN02, DN02, DN03, DN04, DN05, and DN06) have antioxidant activity values of endophytic extracts that are lower than vitamin C activity and are still relatively weak. Even though the antioxidant activity of N. fruticans *is classified as very weak, but at all concentrations the treatment of the isolate has the ability as an antioxidant and method optimization is needed in order to obtain a good antioxidant value*

Introduction

Mangroves are one of the rare and unique ecosystems in the world, because they cover only 2% of the earth's surface. Indonesia is the largest mangrove ecosystem in the world. Jambi Province is one of the provinces in Indonesia which has a mangrove ecosystem area. This ecosystem has a very important ecological, socio-economic and socio-cultural role. According to Burhanuddin (2016) Mangrove ecosystems are typical types of ecosystems located along beaches or river mouths that are affected by tides. Mangroves grow on protected or flat beaches, usually along the windward side of the island or behind coral reefs that are protected offshore.

The mangrove ecosystem, which is a transitional ecosystem between land and

sea, has long been known to have an important role in life and is a very important chain in maintaining the balance of biological cycles in water. *Nipah* or *Nypa fruticans* is member of the Palmae tribe which grows along rivers that are affected by tides and this plant is also grouped in the mangrove forest ecosystem (Suedy et al., 2015).

Palm trees are often used by the community to make roofs and craft materials, while its fruit is rarely used by the community. According to Subiandono and Heriyanto (2011) Nipah is also a source of food and energy, but has not been widely publicized regarding to its potential or utilization. *Nipah* plant parts have also been used as a traditional medicinal ingredient such as stomachaches, diabetes and heat-lowering drugs by the coastal communities of the Banyuasin district in South Sumatra. The coastal communities of Aceh believe that the boiled water of the palm leaves is effective as a medicine for sores cancer and toothaches. Boiled charcoal root water and palm leaves in Kalimantan are used as a remedy for toothaches and headaches (Mangrove Information Center 2009).

Previous research found that *nipah* plants contain antioxidant and antibacterial active ingredients. Putri et al. (2013) showed that crude extracts of nipah leaves with polar methanol 1: 5 (w / v) had an IC50 value of 17.72 ppm. Effendi and Suhardi (1998) stated that *Nypa fruticans* has antibacterial activity against *Vibrio harveyi* in tiger shrimp (*Penaeus monodon*). Lestari et al. (2016) found the zone of inhibition of ethyl acetate fraction of *nipah* leaf extract against *Eschericia coli* (9.39 mm) and *Bacillus cereus* (9.01 mm) at a concentration of 1000 ppm. Active compounds that generally play a role in antioxidant activity are tannins, flavonoids, phenolics, saponins, and terpenoids. Margaretta et al. (2011) states that phenolic compounds have hydroxyl groups in their molecular structure that have free radical scavenger activity and if the hydroxyl group is more than one, the antioxidant activity is stronger.

Ajizat (2004) said that flavonoids, saponins, terpenoids, phenolics and tannins compounds are also active compounds that function as antimicrobial compounds. *Nypa fruticans* is thought to contain components that have antioxidant and antibacterial activity. The antioxidant activity possessed by *nipah* plants may also be owned by endophytes that are in the *nipah* tissue and have the opportunity to produce better antioxidants.

Endophytes are associations of microorganisms that live in plant tissues in fruit, seeds, roots, stems or other parts. Endophytes usually produce compounds and have the same active ingredients as plants producing active substances that have potential as antioxidants and certain other properties. The potential of these endophytic microbes is an abundant natural resource that can be used as a source of new drug discovery. The lack of information about antioxidant activity from *nipah* endophytes from Tanjung Jabung Timur Jambi is interesting to be studied. Therefore, this study was conducted on the isolation of *N. fruticans* endophytic fungi from Tanjung Jabung Timur and to test its activity as an antioxidant.

Materials and Methods

Nipah sampling (*Nypa fruticans*)

Nipah (N. fruticans) sample is leaves that were obtained from the Nipah Panjang mangrove forest area of Tanjung Jabung Timur District. The healthy, shiny and good leaves in the order of 3-5 of the shoots was taken carefully. The leaves are placed in a *mobicool* at 4° C to keep it fresh until it processed in the Laboratory.

Endophytes Isolation from *Nipah (Nypa fruticans*)

Prior isolation of endophytic fungi, the surface of *N. fruticans* leaf was sterilized by disinfecting to kill microbial contaminants. The method of leaf surface sterilization follows the Kumaran and Hur

(2009) procedure: leaves are washed with running water. 0.5 cm2 leaves were cut and sterilized sequentially using 70% alcohol, 5.25% NaOCl and sterile distilled water for one minute each. The surface of the leaves is dried using sterile tissues and isolated on PDA media.

The Production of Secondary Metabolite of *Nipah (Nypa fruticans*)

In producing secondary metabolites, the method used follows Putri et al., 2013, some modifications have been made. Secondary metabolites of endophytic fungi are obtained by fermentation. Previously the fungi were conditioned at room temperature 27° C for 3-7 days depending on the speed of growth on the PDA medium.

Five plugs of 5 mm diameter endophytic fungi were cultivated in Potato Dextrosa Broth, pH 7.2 - 7.4 for 1 L aquades. The size of the isolate used as an inoculum follows the Kumaran & Kour (2009) method. Previously, the inoculum was inoculated for 3-7 days as a starter. 10% v/v volume of the media was cultivated and shakered at 200 rpm to speed up the lag phase. Each sample was replicated 3 times. One tube that was not given an inoculum was used as a control. The culture was fermented for 21 days under static conditions, with 12 hours of light cycle and 12 hours of dark cycle (Gangadevi & Muthmuary, 2008).

The crude extract is obtained by centrifugation of fermentation for 10 minutes at 4000 rpm at -4° C. The culture was filtered using filter paper and the mycelium was removed. The supernatant was extracted with n-hexane (nonpolar), allowed to stand for 24 hours, then filtered. The filtrate is accommodated and the residue is re-maceration in the same way for three times, until the filtrate is clear. The combined filtrate obtained was concentrated with a vacuum evaporator at 60^{o}C until a concentrated (solvent-free) extract was obtained. The residue is dried at room temperature until it is solvent free. In the same way the residues were successively macerated again with 300 mL ethyl acetate (semi-polar) and with methanol (polar). Furthermore, each concentrated extract obtained was carried out an antioxidant test at a concentration of 1000 ppm with the DPPH method in knowing which fraction showed the highest activity.

Antioxidant Activity Test

The antioxidant activity test using the DPPH method following the method of Putri et al., 2013 was carried out by making concentrated extracts of n ethyl acetate, and methanol in endophytic *nipah* plants to a concentration of 1000 ppm. The procedur is by weighing 4 mg of concentrated extracts dissolved into 4 mL of solution in DMSO. DPPH solution was prepared with a concentration of 0.05 mM, by dissolving 1.98 mg of DPPH in 100 mL of methanol. Solution of each sample of 0.2 mL was added 3.8 mL of 0.05 mM DPPH solution. The mixture of solutions is homogenized and left for 30 minutes in a dark place. Uptake was measured by a UV-Vis spectrophotometer at λ 517 nm. Vitamin C (ascorbic acid) is used as a positive control of antioxidants. Selected extracts that had the highest activity at this concentration were continued using series at concentrations of 500, 250, 125, 62.5, 31.25 and 15,625 ppm.

Data analysis

Endophytes that have antioxidant activity are determined by the amount of DPPH radical absorption barrier based on the percentage of absorption inhibition

$$\% \text{ Inhibisi} \quad \frac{Ak - As}{Ak} \quad X\ 100\ \%$$

Extracts that had the highest activity were calculated IC_{50} values (50% inhibition consentration) using a linear regression equation.

Results and Discussion

From the isolation of *Nypha fruticans*, 6 isolates of endophytic fungi were obtained with the characteristics shown in Figure 1 and Table 1.

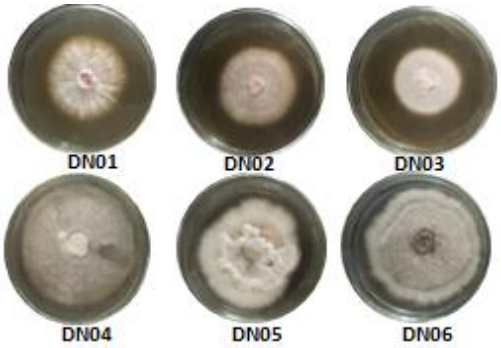

Figure 1. Macroscopic Characteristics of *N. fruticans* Endophytic Fungus Isolates, DN 01 = Isolate 1, DN02 = Isolate 2, DN03 = Isolate 3, DN 04 = Isolate 4, DN05 = Isolate 5 DN06 = Isolate 6

N.fruticans are plants that adapt to aquatic environments that are affected by brackish water (Jayvee et al., 2016). Soil physicochemical properties of *N. fruticans* according to Rasco et al., (2011) are unique compared to the plant environment generally because the soil has a low level of phosphorus and nitrogen content in saline conditions that still survive and have high productivity. As reported by Tang et al., (2010) it has been found that the endophytic *Burkhodlderia vietnamiensis* associated with *N. fruticans* plays a role in nitrogen fixation. Choo, J., et al., (2015) also found that endophytic Pestaliopsis in *N. fruticans* from Kuching Sarawak National Park in Malaysia was able to withstand the accumulation of heavy metals such as (Cu), iron (Zn), lead (Pb), chromium (Cr), thus acting as a bioremediation agent. Jayvee et al., (2016) reported that microbes associated with *N. fruticans* also have the potential to induce PGPR compounds that trigger plant growth.

Table 1. Macroscopic Characteristics of *N. fruticans* Endophytic Fungi

Character	Isolate					
	DN 01	**DN 02**	**DN 03**	**DN 04**	**DN 05**	**DN 06**
Top surface	White	White	White	White	Grayish	Grayish
Bottom surface	White	White	White	White	Brown yellowish	Blackish
Texture	Cotton	Cotton	Cotton	Cotton	Cotton	Cotton
Margin	Plain	Plain	Plain	Plain	Plain	Plain
Pattern	Spread	Spread	Spread	Spread	Spread	Spread
Growing speed	Low	Low	Fast	Fast	Fast	Low

N.fruticans Endophytic Antioxidant Activity

Analysis of antioxidant activity was seen through IC_{50} values using DPPH. DPPH is a free radical that is stable and capable of receiving H atoms from anti-oxidants. Research on *N. fruticans* by Prasad et al., (2013) has concluded that ripe and young fruit of *N. fruticans* can inhibit DPPH free radicals with IC_{50} values of 70ppm and 85ppm respectively which are categorized as having moderate antioxidant activity. Yusoff, NA et al., (2015) also reported that ethyl acetate extract of *N. fruticans* was also potential in inhibiting free radicals. In this study IC_{50} values were calculated using methanol and ethyl acetate solvents (Table 2) and vitamin C was used as a comparison. IC_{50} value of vitamin C in methanol was 25ppm while in ethyl acetate the value was 395 ppm.

Table 2. The antioxidant activity of *N.fruticans* endophytes in Methanol and Ethyl acetate solvents

Treatment	IC_{50} (ppm)		Antioxidant character	
	Metanol	Etil Asetat	Metanol	Etil asetat
DN01	6030	7029	Weak	Weak
DN02	34470	6668	Weak	Weak
DN03	16630	7236	Weak	Weak
DN04	10777	-7865	Weak	Weak
DN05	5904	2415	Weak	Weak
DN06	1323	4435	Weak	Weak

Table 2 shows that the antioxidant activity value of endophytic extracts is lower than vitamin C activity and is still relatively weak in both solvents. Although *N. fruticans* endophytes are classified as very weak, *N. fruticans* at all concentrations of treatment still has antioxidants and optimization methods are needed to obtain good antioxidant values.

Conclusion

1. Six isolates (DN01, DN02, DN02, DN03, DN04, DN05, and DN06) of endophytic fungi have been successfully obtained from Nypha fruticans from Tanjung Jabung Timur
2. The six isolates have antioxidant activity, with IC_{50} values > 50% which are categorized as weak.

Acknowledgment

The author would like to thank the University of Jambi DIPA 2018 for funding this research.

References

Ajizat A. 2004. Sensitivitas *Salmonella typhirium* terhadap ekstrak daun Psidium Guajava. *Journal Bioscientive* 1: 31-38.

Burhanuddin. 2016. Kajian Kondisi, Potensi dan Pengembangan Hutan Mangrove di Kabupaten Serdang Bedagai. *Wahana Inovasi*. 5(2): 482-490

Choo, J., Nuraini B.M.S., Daniel T, Aazani M, and Moritz M.2015. Heavy Metal Resistant Endophytic Fungi Isolated from Nypa fruticans in Kuching Wetland National Park. *Ocean Sci. J*. 50 (2):445-453

Gangadevi, V & Muthumary. J. 2008. Isolation of a Novel Endophytic Taxol-Producing Fungus from the Leaves of a Medicinal Plant *Justicia gendarussa. Mycologia Balcanica*. 5: 1-4

Jayvee, A. C. Katreena, M. Cadlente, M. 2016. Assesment of Potential Plant Growth Promoting Compound Produced Invitro by Endophytic Bacteria assosiated wuth Nipa Palm (*Nypha fruticans*). *Philipine Journal of Crop Science* (PJCS). 41 (3): 74-82

Kumaran, R. S *and* Hur, B.K. 2009. Screening of Species of the Endophytic Fungus *Phomopsis* for the Production of the Anticancer Drug Taxol. *Biotechnol. Appl. Biochem*. 54: 21–30

Lestari, Y. Ardiningsih,P., Nurlina. 2016.Aktivitas Antibakteri Gram Positifdan Negatif Dari Ekstrak dan Fraksi Daun Nipah (*Nypa fruticans* Wurmb.) Asal Pesisir Sungai Kakap Kalimantan Barat. *JKK*. 5(4): 1-8I.

Mangrove Information Center [MIC]. 2009. *Nypa fruticans*. Bali. Denpasar. www. mangrovecenter.or.id

Margaretta S dan Handayani SD. 2011. Ekstraksi Senyawa Phenolik *Pandanus amaryllifolius* ROXB sebagai Antioksidan Alami. *Jurnal Widya Teknik* 10: 21-30.

Prasad N, Yang B, Kong KW, Khoo HE, Sun J, Azlan A, Ismail, A, Romli ZB. 2013. Phytochemicals and Antioxidant Capacity from *Nypa fruticans* Wurmb. Fruit. *Evid Based Complement Alternat Med*. 13: 1-9

Tang, SY. Hara S, Melling L, Goh KJ, Hashidoko Y. 2010. *Burkholderia vietnamiensis* Isolated from Root Tissues of Nipa Palm (*Nypa fruticans*) In Sarawak, Malaysia, Proved To Be Its

Major Endophytic Nitrogen-Fixing Bacterium. *Biosci Biotechnol Biochem*. 74:(9):1972-5

Putri IJ, Fauziyah, Elfita. 2013. Aktivitas Antioksidan Daun Dan Biji Buah Nipah (*Nypa fruticans*) Asal Pesisir Banyuasin Sumatera Selatan dengan metode DPPH. *Maspari Journal*. 5(1): 16-21.

Rasco ET Jr. 2011. The Nypa Palm: Nature's Gift from the Age of Dinosaurs. National Academy of Science and Technology, Bicutan, Taguig, Metro Manila, Philippines

Subiandono E., and Heriyanto NM. 2011. Potensi Nipah (*Nypa fruticans* (Thumb.) Wurmb.) Sebagai Sumber Pangan dari Hutan Mangrove. *Buletin Plasma Nutfah* (17) (1):54-60

Suedy SWA., Soeprobowati TR., Hidayat JW. 2015. Dinamika *Nypa fruticans* Wurmb. Di Muara Banjir Kanal Timur Semarang Berdasarkan Bukti Polennya. Buletin Anatomi dan Fisiologi. 23(1):

Xiong, Z.,Yang, Y., Zhao, N & Wang, Y. 2013. Diversity of Endophytic Fungi and Screening of Fungal Paclitaxel Producer from Anglojap Yew, Taxus x Media. *BMC Microbiology*. 1: 1-10.

Yusoff NA, Yam MF, Beh HK, Abdul Razak KN, Widyawati T, Mahmud R, Ahmad M, Asmawi, MZ. 2015. Antidiabetic and Antioxidant Activities of *Nypa fruticans* Wurmb. Vinegar Sample from Malaysia. *Asian Pac J Trop Med*. 8 (8):595-60