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DIVERSITY OF MICROBIAL ENDOPHYTES IN THE STEM AND STEM WATER OF *Ceiba pentandra*

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ABSTRACT

Ceiba pentandra (Kapok trees) serve the purpose of drug which we know as tribal medicine. Based on research before, most of secondary metabolites were found in the stem and stem water of *C. pentandra* which could produce antimicrobial compounds against *Staphylococcus aureus* and *Escherichia coli*. Nevertheless, little is known about the diversity of endophytic microorganisms inhabit the stem and stem water of *C. pentandra* and their potential to produce bioactive compounds. The purpose of this work was to know the diversity of endophytic microorganisms inhabited the stem and stem water of *C. pentandra*. The microbes were isolated from the stem and stem water of *C. pentandra*. The endophytes were characterized based on the morphology appearance of the microbial endophytes microscopical and macroscopical. From the samples of stem water, 278 isolates of the microbial endophytes grew at NA medium and 31-257 isolates at PDA medium whereas from stem, 4 isolates of the microbial endophytes grew at NA medium and 2 isolates at PDA medium. When identified morphologically and biochemically, only 7 colonies at stem water and 5 colonies at stem had showed identifiable differences. Among the microbial group the most frequently in endophytic association with stem water may it be khamir. Meanwhile, bacteria was the most frequently in endophytic association with stem. This research indicated that culturable endophytes in the stem and stem water of *C. pentandra* were very diverse. Microbial endophytes are suggested as an outstanding source of bioactive natural products that could be useful for medicines.

Keywords: *Ceiba pentandra*, microbial endophytes, diversity

INTRODUCTION

Ceiba pentandra (Bombacaceae) commonly called Kapok tree or silk cotton (English), Rimi (Hausa, Nigeria), Vamber (Tiv, Nigeria), King (Yandang, Nigeria) is grown chiefly in Asia and Indonesia. *C. pentandra* is an important product of Java (Doughari & Loryue 2009). *C. pentandra* originated in the American tropics. Its natural distribution has been obscured by its widespread introduction after about 1500. It is now cultivated all over the tropics, but mainly in South-East Asia, especially in Indonesia and Thailand (Probase Record).

C. pentandra finds wide application in Indonesia especially Banten. The root and stem barks are credited with emetic and antispasmodic properties. Stem bark decoctions are used in mouth washes for treating toothache and mouth problems, and are taken to treat stomach problems, diarrhoea, hernia, gonorrhoea, heart trouble, oedema, fever, asthma and rickets; they are also applied on swollen fingers, wounds, sores, furuncles and leprous macules. Leaf preparations are used as an eye-bath to remove foreign bodies from the eye. A decoction of the leaves and water of the stem is applied to treat conjunctivitis and wounds in the eye, and is used for bathing and massage to treat fever (Probase Record).

Secondary metabolites occur in plants in a high structural diversity. Biochemical and physiological features of secondary metabolism are strongly correlated with its function. Secondary metabolites are not useless waste products but important means of plants for de-

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fense against herbivores, microbes (bacteria, fungi) and viruses. Land plants have evolved secondary metabolites with a wide repertoire of biochemical and pharmacological properties (Wink, 2011).

Numerous natural products from *C. pentandra* show striking structural similarities to known secondary metabolites. *C. pentandra* shows the presence of many chemical constituents which are responsible for varied pharmacological and medicinal property (Elumalai *et al*. 2012). Based on Anosike *et al*. (2012), the methanol extract of *C. pentandra* stem bark showed activity against all test isolates with highest activity against the fungi (*Candida albicans* and *Aspergillus niger*) and also had a higher activity on the Gram positive bacteria as compared to Gram negative bacteria. The ethanol and the petroleum ether extract of that had a higher activity on *Escherichia coli* and *Bacillus subtilis* and minimal activity on the fungi for the ethanol extract. Meanwhile, Bairwa *et al.* (2011) had reported the protective activity of ethyl acetate fraction of methanol extract of stem bark of *C. pentandra* against paracetamol-induced liver damage in rats. The phytochemical studies of the methanolic extract revealed the presence of saponins, flavonoids, tannins, terpenes, resins and carbohydrates (Sule *et al.* 2009).

However, production of secondary metabolites from plants is not always satisfactory. It is often restricted to a limited species or genus, and geographically to a specific region. Many important medicinal plants were endangered by overexploitation (Manuhara, 2011). As like *Ceiba pentandra*. This plant is difficult to cultivate and grow very slowly or are endangered in their natural habitats. To avoid that, many efforts for getting the secondary metabolites without overexploitation. An alternative effort for the production of plant materials for secondary metabolite production is the exploration and isolation of the endophytic microbial.

Every plant-species constitutes a possible host for endophytic microorganisms, which, in the vast majority and despite their biotechnological potential (Ezra *et al*. 2004), remain unidentified. Nevertheless, little is known about the diversity of endophytic microorganisms inhabit the stem and stem water of *C. pentandra* and their potential to produce bioactive compounds. It is therefore interesting to investigate the antimicrobial activity of the endophytic microbial of the part of plant especially stem and stem water against some pathogenic bacteria. The aim of this research was to know the diversity of endophytic microorganisms inhabit the stem and stem water of *C. pentandra*.

MATERIALS AND METHODS

The samples of *C. pentandra* were collected from the village around Lebak district, Banten. The bark was removed manually and dried, debarked logs were prepared into chips of suitable size that remain between 2-5 mm. For getting stem water, stem were cut using sterile machete and taken to the laboratory in sterile containers at temperature of 4 °C. The endophytic microbial of the *C. pentandra* was screened and characterized based on the morphology appearance of the endophytes microscopical and macroscopical. Purified strains were characterised using morphological and biochemical tests according to standard methods: catalase test and oxidase test. Cell morphology, gram stain and motility were observed using light microscopy.

Prior to analysis, each sample was tested againsts the indicator strains of bacteria, they are *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*. The agar well diffusion method was performed to exploit antibacterial potential (Bibi *et al*. 2010). Each endophytic microbial was tested the viability of antibacterial activity for *E. coli*, *S. aureus*, and *B. cereus*. Boric acid 10% (5.7 g/ 100 mL) in aquadest was prepared as positive control. Pure DMSO (99.9%) was used as negative control. Drops of culture (48 hours for bacteria and 4-5 days for fungi) of each tested strain were spotted onto agar plates seeded with active growing cells of the indicator organism. After incubation (24 hours at 37 ºC), the antimicrobial activity was determined as a clear zone of inhibition around the spots. Isolates showing ability to produce antibacterial compounds were chosen for further analysis.

RESULTS AND DISCUSSION

Endophytic microbial were isolated from different parts of *C. pentandra*. The microbial population of stem water ranged from 2.78×10^6 to 1.23×10^8 CFU.g^{-1} on the NA plates and from 31 to 257 on the PDA plates (Table 1). Among all the isolates on the NA plates and the PDA plates, colonies were chosen according to their morphological differences on each NA plate and PDA plate. More colonies were chosen from the higher population of culturable bacteria on each of the NA plates and PDA plates. The total of endophytic microbial densities recorded from the surface of the *C. pentandra* indicate slight differences in the numbers of viable bacteria isolated from different parts (stem and stem water). The number of endophytic microbial that isolated from stem water are higher than those isolated from stem, which could suggest that the endophytic microbial more inhabit the stem water than the stem of *C. pentandra*.

Table 1. Endophytic microorganisms that isolated from the stem and stem water of *Ceiba pentandra*

The type of samples	The type of medium	The total of colonies	The total of isolates	The group of microbes	Notes
Stem	NA	278	3	bacteria	B1, B2, B3
Water	PDA	31-257	4	khamir	SP4, SP6, SP7, SP9
Stem	NA	4	4	bacteria	BN1, BN2, BN3, BN4
	PDA	2	1	kapang	BP1

The endophytic microbial of the *C. pentandra* was found in stem and stem water. At the stem water, four of which were identified as khamir, three of which were identified as bacteria. At the stem, four of which were identified as bacteria and one of which was identified as kapang. Endophytic communities are formed mainly by fungi and bacteria. Although the interaction between these microorganisms and their respective host-plants is not, as yet, fully understood, over recent years they have been progressively more extensively employed, either in agriculture (Ryan *et al*., 2008), or in the production of compounds with therapeutic application, such as taxol (Stierle *et al*., 1993) and leucinostatin A (Strobel & Hess 1999). Literature data reporting, 16 isolates of the endophytic microbial were obtained at *C. pentandra*, although, when compared to sequences deposited in GenBank, only seven had produced identifiable fragments. They are *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus* sp. that isolated from the seed of *C. pentandra* (Coelho *et al*., 2011).

The endophytic microbial from *C. pentandra* was assayed for their antimicrobial activity against the indicator strains of bacteria. Antimicrobial activity of endophytic microbial from stem and stem water of *C. pentandra* was found in several isolates (Table 2). At the stem water, two of khamir (SP6 and SP9) and bacteria (B2 and B3) have antibacterial activity. At the stem, all of bacteria isolates have antibacterial activity. One strain of khamir obtained from the stem water (SP6) only produced antibiotic compounds active against *E. coli* whereas one strain of that (SP9) produced antibiotic compounds active against *S. aureus* and *B. cereus*. Different with bacteria, one strain of bacteria (B2) produced antibiotic compounds active against both *E. coli* and *B. cereus* isolates while the strain of B3 only produced antibiotic compounds active against *B. cereus*. Meanwhile, the strains of bacteria that obtained from the stem of *C. pentandra*, like BN2 have a clear zone of inhibition for *S. aureus*; BN4 for *E.coli* and *B.cereus*; and BN3 for all of indicator strains of bacteria.

Table 2. The test of antibacteria activity from endophytic microorganisms that isolated

The type of samples	The type of isolates	The group of microbes	The zones of inhibited (cm)		
			E. coli	*S. aureus*	*B. cereus*
Stem Water	SP4	Khamir	0	0	0
	SP6		0.8	0	0
	SP7		0	0	0
	SP9		0	0.7	0.8
	Kontrol (+)		1.3	1.2	1.8
	Kontrol (-)		0	0	0
Stem Water	B1	Bacteria	0	0	0
	B2		0.7	0	0.7
	B3		0	0	0.8
	Kontrol (+)		0.9	1.2	1.0
	Kontrol (-)		0	0	0
Stem	BN2	Bacteria	0	0.7	0
	BN3		0.7	0.7	0.7
	BN4		0.7	0	0.7
	Kontrol (+)		0.9	1.1	0.9
	Kontrol (-)		0	0	0

Strain SP6 formed slow-growing, smooth-rising, small-sized, pink-pigmented colonies. It had a spherical shape of the colony. Strain SP9 formed slow-growing, unsmooth-rising, small-sized, white-coloured colonies. It had a spherical shape of the colony. Strain B2 and B3 was characterised as a Gram-negative, white-coloured colonies, irregular round shape and irregular rod shape. Strain BN2 was characterised as a Gram-negative, regular bumpy round shape, and white-coloured colonies. Strain BN3 and BN4 was characterised as a Gram-positive, white-coloured colonies. They had a irregular round shape and irregular rod shape of the colony.

The SP9 isolates of khamir demonstrated antibacterial activity against both *S. aureus* and *B. cereus* demonstrating a higher activity than another. Meanwhile, the B2 isolates of bacteria demonstrated antibacterial activity against both *E. coli* and *B. cereus*. In this work, BN3 isolates have broad spectrum more of antibacterial activity than another. Strain BN3 revealed an indication that the endophytic microbial of *C. pentandra* contains secondary metabolites that have broad spectrum of antibacterial activity. This is actually a very significant discovery giving hope for the possible development of a novel endophytic from C. pentandra that can be effective in controlling multidrug resistant bacteria and a variety of other bacterial disease agents. The results of antimicrobial activity suggest that SP6, B2,

BN4, and BN3 had the presence of tannins, alkaloids, saponins, flavonoids, sterols and or triterpenes and reducing sugars because antidiarrhoeal properties of medicinal plants were found to be due to that (Longanga *et al*., 2000).

In conclusion, results of this study revealed the presence of the endophytic microbial in the stem and stem water of *C. pentandra*. Endophytic communities are formed mainly by fungi (khamir and kapang) and bacteria. Endophytic microbial of the stem water of *C. pentandra* are higher of the number than the stem. Neverthelles, endophytic microbial of the stem showed broad spectrum more of antibacterial activity than endophytic microbial of the stem water of *C. pentandra*. However, the results of this research could be references more about the diversity of endophytic microorganisms inhabit the part of *C. pentandra*.

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