

PHYTOCHEMICAL TEST AND ANTIBACTERIAL BIOACTIVITY OF EXTRACTS FROM *Artocarpus integer* (Thunb.) Merr Stem Bark

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Abstrak. Penelitian ini bertujuan untuk mengidentifikasi golongan senyawa dan menguji aktivitas antibakteri dari fraksi n-heksan, kloroform, dan metanol kulit batang *Artocarpus integer* (Thunb.) Merr. Semua fraksi mengandung golongan senyawa terpenoid dan steroid. Aktivitas antibakteri ditentukan menggunakan metode difusi agar dengan media MHA (Muller Hinton Agar) terhadap *Escherichia coli* (gram negatif) dan *Staphylococcus aureus* (gram positif). Ciprofloxacin digunakan sebagai kontrol positif dan metanol sebagai kontrol negatif. Ketiga fraksi tersebut mampu menghambat pertumbuhan bakteri *Escherichia coli* dan *Staphylococcus aureus*. Fraksi kloroform menunjukkan aktivitas tertinggi setelah inkubasi 48 jam dengan zona hambat masing-masing yaitu 10.8 dan 10.4 mm.

Kata kunci: Fraksi, *Artocarpus integer* (Thunb.) Merr, Fitokimia, Antibakteri

Abstract. This research aims to identify the compound group and determine the antibacterial activity of n-hexane, chloroform, and methanol fractions of *Artocarpus integer* (Thunb.) Merr stem bark. All fractions contain groups of terpenoid and steroid compounds. Antibacterial activity was determined using agar diffusion method with MHA (Muller Hinton Agar) media on *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive). Ciprofloxacin was used as a positive control and methanol as a negative control. The three fractions were able to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*. The chloroform fraction showed the highest activity after 48 hours incubation with inhibition zones of 10.8 and 10.4 mm respectively.

Keywords: Fractions, *Artocarpus integer* (Thunb.) Merr, Phytochemical, Antibacterial

INTRODUCTION

Moraceae is a tropical plants that have the potential to produce secondary metabolites in relatively large quantities. The *Moraceae* family consists of 75 genus and 180 species (Hakim, 2010). The main genus of the family *Moraceae* is *Artocarpus* which consists of 50 species that spread in the South Asian region, Papua New Guinea, the South Pacific, and Indonesia (Hakim, 2010). *Artocarpus* plants are found in Indonesia with characteristic that is a tall tree with white sap in all parts of the plant, hardwood, fruit with flesh and many seeds. All parts of the *Artocarpus* plant have been used by many people for various purposes, one of which is traditional medicine. The plant *A. camansi* was believed to be able to treat blood sugar and skin drugs through decoction of leaves. The root of *A. heterophyllus* was used to treat fever, dysentery, and malaria, its leaves as a remedy for boils, fever, wounds, and skin diseases. *A. integer* fruit seeds have long been used by the people of Kampar (Riau Province) as a drug to treat diarrhea and for diabetes (Hilma et al., 2018).

Pharmacological studies in vitro and in vivo on the bioactivity of the genus *Artocarpus* have been widely performed, such as antimalarial activity (Boonlaksiri, et al., 2000), antioxidants (Zakaria et al., 2017), anticancer (Ganeson et al., 2017), antiproliverative (Hashim et al., 2012), antidiabetic (Nuntawong, et al., 2017), and

antivirus (Hafid et al., 2017). Most of these pharmacological effects arise because of the content of flavonoids, terpenoids, stylbenoid, arylbenzofuran, neolignan and *adducts* Diels Alder (Hakim, 2010).

Antibacterials are chemical agents capable of inactivating bacteria. Inactivation of bacteria can be in the form of inhibition of bacterial growth (bacteriostatic) and can even kill bacteria (bactericidal) (Pelczar and Chan, 1988). The antibacterial potential in the genus *Artocarpus* can be seen from its minimum inhibitory concentration. Madhavi et al. (2013) reported that methanol extract of *A. heterophyllus* fruit sap had broad spectrum antibacterial activity that could inhibit the growth of *Pseudomonas aeruginosa* and *Escherichia coli*. In the same species, artocarpesin compounds were found which were able to inhibit the growth of *Staphylococcus aureus* (Navarro-Garcia et al., 2012). The antibacterial activity of chloroform extract from *A. integer* stem wood provided a barrier zone of 13.80 mm against the bacterium *Streptococcus pneumonia* (Zakaria et al., 2017). The antimicrobial activity of ethanol extract of *A. lakoocha* bark on five test bacteria is bacteriostatic so that it can be developed as a source of antibiotic substances for drug development and in bacterial control (Nath and Boruah 2019).

The development of antibiotic drugs has special attention to researchers. This is due to the administration of antibiotics as the most appropriate solution for

controlling pathogenic bacteria in infectious diseases. However, currently some antibiotics are no longer effective due to the emergence of resistant pathogenic bacteria. Data shows resistance to conventional antibiotics and it is estimated that in 2050 worldwide, 10 million people per year will die from infection by resistant microbes (Cordes et al., 2014). This condition encourages researchers to find effective new antibacterial agents capable of killing or fighting bacterial growth.

Although it has been known that the *Artocarpus* plant was ethnobotany used as a medicine, the chemical content and bioactivity of *A. integer* (Thunb.) Merr stem bark has not been reported. Therefore, this article will report on phytochemical tests and antibacterial activity with agar diffusion methods.

MATERIAL AND METHOD

Instruments

The instruments used in this study were distillation, rotary evaporator, buchner funnel, funnel, separating funnel, test tube, analytic balance, eppendorf micropipette, autoclave, aluminium foil, paper disk, petri dish, incubator, calipers, bunsen, lamp and glassware generally used in laboratories.

Materials

The materials used in this research were the stem bark powder of *Artocarpus integer* (Thunb.) Merr, organic solvents such as technical methanol, technical n-hexane, chloroform p.a, Liebermann-Buchard reagent, Dragendorff, Wagner, iron (III) chloride, pure culture of

Escherichia coli and *Staphylococcus aureus*, Nutrient Agar (NA) and Muller Hilton Agar (MHA) media, DMSO, ciprofloxacin, disc paper, aquadest, spiritus, 70 % alcohol, aluminum foil, cling wrap and tissue roll.

Methods

1. Sample collection

Stem bark of *Artocarpus integer* (Thunb.) Merr was collected in September 2017 in Harapan Village, Mappedeceng District, North Luwu Regency, South Sulawesi. Plant identification was done at Herbarium Bogoriense, Biology Research and Development Center, LIPI Bogor (Zakaria, 2017)

2. Extraction and phytochemical tests

A total of 3 kg of bark powder macerated with methanol for 3 x 24 hours, then filtered to get the extract. The extract was concentrated using a rotary evaporator and then partitioned with several solvents to obtain the n-hexane, chloroform, and methanol fractions. All fractions were tested for phytochemical and antibacterial activity. Phytochemical tests included alkaloid test with Meyer and Wegner reagent, terpenoid test with Liberman-Burchard reagent, steroid test with Liberman-Burchard and Salkowski reagents, flavonoid test using Mg and concentrated HCl, and phenolic test with FeCl₃ reagent.

3. Antibacterial Activity Test

Antibacterial activity tests were carried out on all fractions (n-hexane,

chloroform and methanol) using agar diffusion methods, with variations in concentrations of 100, 250, 500 ppm respectively. Ciprofloxacin was used as a positive control and methanol as a negative control. Incubation was carried out for 48 hours, and the clear zone was measured every 24 hours.

RESULTS AND DISCUSSION

1. Phytochemical Test

The result of the phytochemical test showed that the three fractions contained terpenoid and steroid compounds (Table 1).

Table 1. Phytochemical Test Results of n-Hexane, Chloroform and Methanol Fraction of *A. integer* (Thunb.) Merr Stem Bark

No.	Test	Reagents	Result		
			n-Hexane	Chloroform	Methanol
1	Flavonoid	Mg + HCl Concentrated	-	++	++
2	Terpenoid	Lieberman-Burchard	++	++	+
3	Steroid	Salkowski	+	+	+
4	Alkaloid	Wegner	+	-	-
		Meyer	+	-	++
5	Phenolic	FeCl ₃ 10 %	-	++	+++

The phenol and flavonoid compounds were founded in the chloroform and methanol fractions. The group of alkaloid compounds obtained in the n-hexane and

methanol fractions. This is following the previous work reported by Hilma et al. (2018) against *A. integer* (Thunb.) Merr with fruit seed extract.

2. Antimicrobial Test

The results of the measurement of inhibition zones of n-hexane, chloroform, and methanol fractions against *E. coli* and *S. aureus* are shown in Table 2.

Table 2. Antibacterial Activity of n-Hexane, Chloroform, and Methanol Fractions from *A. integer* (Thunb.) Merr Stem Bark Against *E. coli* and *S. aureus*

Fractions	Inhibition zone (mm)			
	<i>E. coli</i>		<i>S. aureus</i>	
	24 hours	48 hours	24 hours	48 hours
n-Hexane	9.0	9.7	9.7	9.5
Chloroform	9.4	10.8	9.8	10.4
Methanol	8.9	10.1	9.4	9.7
Negative Control (Metanol)	6.0	6.0	6.0	6.0
Positive Control (Ciprofloxacin)	32.3	17.9	18.5	33.7

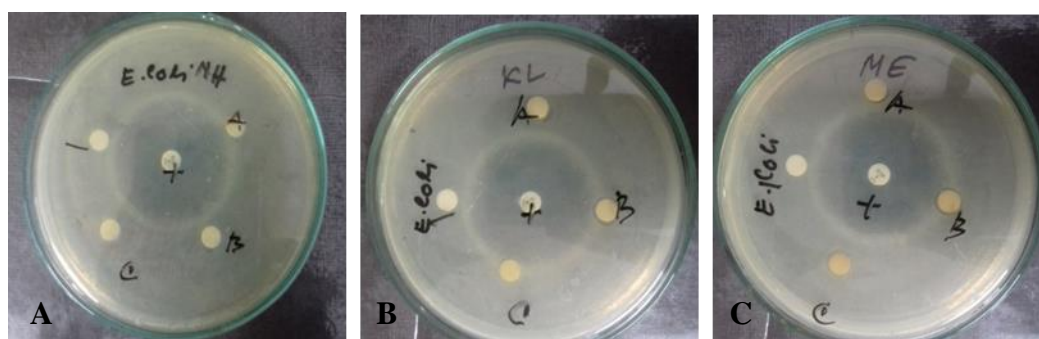


Figure 1. Inhibition zone for *Escherichia coli*, **A.** n-Hexane fraction; **B.** Chloroform fraction; **C.** Methanol fraction.

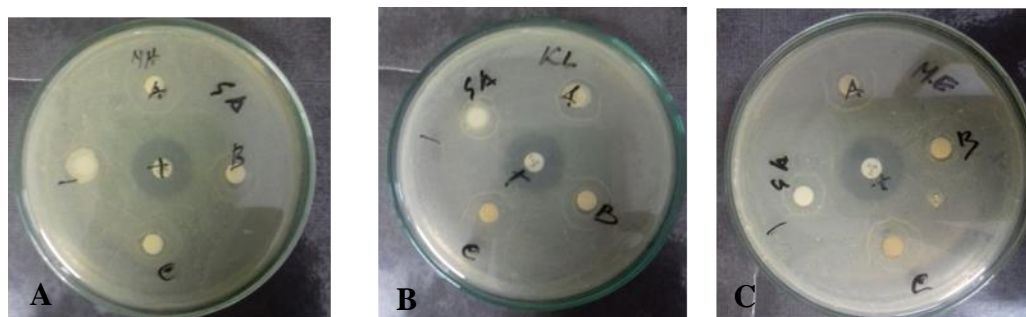


Figure 2. Inhibition zone for *Staphylococcus aureus*, **A.** n-Hexane fraction; **B.** Chloroform Fraction; **C.** Methanol fraction.

Generally n-hexane, chloroform, and methanol fractions can inhibit the growth of *E. coli* and *S. aureus* bacteria (Table 2).

The highest activity of each fraction was obtained at a concentration of 250 ppm. The fraction that gave the best inhibition

zone to both bacteria was obtained in the chloroform fraction.

According to (David and Stout, 1971) inhibition zones of 5-10 mm was a moderate inhibition response. Ciprofloxacin provides the highest inhibition zone, when compared to the three stem bark fractions of *A. integer* (Thunb.) Merr. In gram negative bacteria, ciprofloxacin is bacteriostatic, whereas in gram positive is bactericidal (Pelczar and Chan, 1988).

CONCLUSION

The n-hexane, chloroform, and methanol fractions of *A. integer* (Thunb.) Merr stem bark contains compound groups of terpenoids and steroids. Chloroform fraction has the potential to be developed as an antibacterial because it has the highest inhibitory power (10.80 and 10.40 mm) at 48h incubation time on the growth of *E. coli* and *S. aureus*.

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