

Phytochemical Screening and Antimicrobial Activity of *Xylocarpusgranatum* J.Koenig leaves

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ABSTRACT: *Xylocarpusgranatum* J. Koenig is a traditional Indonesian plant known as “Nyireh”. The antimicrobial activity of the ethyl acetate and n-hexane fraction extract of Nyireh’s leaves was evaluated against Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 9027), and fungus (*Candida albicans* ATCC 10231). The extracts were screened for their antimicrobial activity using Kirby-Bauer Disc Susceptibility Test. Both extracts inhibited the growth of *Staphylococcus aureus* (15.2 mm) and *Pseudomonas aeruginosa* (10.7 mm), and both fractions did not have antifungal activity against *Candida albicans*. The phytochemical analysis detected the presence of alkaloids, flavonoids, saponins, tannins, and terpenoids. The findings of the study may be helpful to future investigators in identifying alternative and new bioactive secondary metabolites like antibiotics to treat resistant human pathogens.

KEYWORDS: *Xylocarpusgranatum* J. Koenig, Phytochemical screening, Antimicrobial activity, Kirby-Bauer Disc Susceptibility Test

I. INTRODUCTION

Traditional medicine is used by a large proportion of the Indonesian population as the major health need of humans and animals. *Xylocarpusgranatum* J. Koenig is a well-known traditional plant of Indonesia, it is popularly recognized as “Nyireh”. With the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for bioactive compounds is important and has been confirmed for traditional medicinal uses. In Indonesia, this plant uses for dysentery, cholera, diarrhea, febrifuge, and skin infections. The bark extract showed antibacterial activity and antifungal activity [1]. The leaves are used traditionally for the treatment of shortness of breath by drinking boiled water from the leaves [2]. Thus, the objective of the present study was intended to

evaluate the phytochemical screening and antimicrobial activity of Nyireh.

II. MATERIAL AND METHODS

Sample collection and preparation

The plant material was collected randomly taken from the island of BulangLintang Batam, Riau Islands Indonesia. It was identified by the Department of Biology at Andalas University Padang Indonesia. The fresh leaves of Nyireh were cleaned thoroughly by using tap water, dirt particles were removed and they were shade dried for 5 days. After drying, the leaves were ground into a fine powder. The powder was stored in an air-tight container for further use. 300 grams of the powdered plant material was extracted successively with ethanol 70%, and its fraction of N - hexane, and ethyl acetate fractions were obtained from the liquid-liquid partition of ethanol from the *Xylocarpusgranatum* J. Koenig leaves

Phytochemical Analysis

Qualitative phytochemical analysis of the crude extracts of the plants collected was determined by the methods of screening [3-4]

Antimicrobial screening

The test organisms (*Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, and *Candida albicans* ATCC 10231). Stock cultures were maintained on nutrient agar slants at 4°C and subcultured in nutrient broth at 37°C before each antimicrobial test.

The extracted sample was tested for their antibacterial activity using Kirby-Bauer (KB) method against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. In this test, bacteria culture solutions contain turbidity standard of 0.5×10^6 CFU/ml prepared in normal saline and spread on sterile Nutrient agar plates using the spread plate technique. Then discs containing different concentrates (500, 400, and 300 mg/mL) were placed on an agar plate where

the selected bacteria were grown. Tetracycline and Ketoconazole (10µ g/ml) were used as standard antibacterial and antifungal agents. The plates were kept for 1hr for pre-diffusion and incubated at 37°C/24hr (plates containing bacterial cultures), and 25°C/3days (plates containing *Candida albicans* culture). After incubation, the zone of inhibition was recorded. The experiment was repeated three times.

III. RESULT AND DISCUSSION

Phytochemical analysis

The extractive values for ethanol, n-hexane, and ethyl acetate extracts were found to be 13.65%, 17.23%, and 15.075% respectively. The photochemical analysis of *Xylocarpusgranatum* J. Koenig leaves revealed the presence of alkaloids, flavonoids, saponins, tannin, and terpenoid in the ethanol 70%, an alkaloid in the n-hexane, and alkaloid, flavonoids, saponins, and tannin in the ethyl acetate (Table 1).

Table 1. Qualitative phytochemical screening of *Xylocarpusgranatum* J. Koenig leaves

Active principle	Test	Ethanol 70%	N Hexane	Ethyl acetate
Alkaloid	Mayer	+	+	+
Flavonoid	Mg, HCL conc.	+	-	+
Saponin	Froth test	+	-	+
Tannin	FeCL ₃ 1%	+	-	+
Terpenoid	Acetic acid, H ₂ SO ₄ conc.	+	-	-

According to [5] alkaloids can interfere with the integrity of the peptidoglycan component of bacterial cells, and flavonoids can bring destruction to the bacteria as they could cause the membrane to leak out cell material. Saponin's role as an antibacterial agent in this plant might be because it can cause cell wall malfunctioning. The cell membrane activity will be limited and this will cause the permeability of the membrane to be destroyed which will lead to the malfunctioned cell wall. Tannins were recorded to be able to exhibit inhibition activity on the cell membrane thus disrupting the normal functioning of essential enzymes and genetic material. Bacterial cell wall inhibition by tannins will be done because they will bind to the cell wall. Terpenoids are the largest and the most diverse class of plant compounds and they have numerous functional roles in metabolism and ecology. The antifungal activity of flavonoids may influence by the inhibitory action of fatty acid synthase during the pathway of a gene. Saponins on the other hand will cause the breaking down of membrane integrity by the pore formation on the

fungal membrane because of the complexation that happens between saponins and sterols [6].

Tannins are widely known for their ability to bind to different membrane structures and for their antifungal activity, tannins have the affinity of binding to ergosterol thus reducing the amount of ergosterol in fungal cells [7].

Antimicrobial screening

Table 2 summarizes showed inhibition in both *S. aureus* and *P. aeruginosa* of the ethyl acetate extracts, whereas no inhibition in the n-hexane extracts. This might be due to the sensitivity of the extract depending on the level of the concentration when it is used against certain microorganisms. Ethyl acetate's extract gave a higher inhibition zone compared to n-hexane's extracts, because of the influence of polarity of ethyl acetate's extract being higher than n-hexane's extracts. The *Candida albicans* showed no inhibition of both extracts. From the findings, *S. aureus* was highly inhibited by ethyl acetate extracts, indicates is a relatively strong antibacterial.

Table 2. Zone of Inhibition from Different Concentrations for Each Extract

Microbes	Concentration	500 mg/mL	400 mg/mL	300 mg/mL	Chloramphenicol/ Ketokonazole	DMSO
S. aureus ATCC 6538	N-hexane	0 mm	0 mm	0 mm	41.7 mm	-
	Ethyl acetate	15.2 mm	12.7 mm	10.7 mm	43.3 mm	-

P. aeruginosaATCC 9027	N-hexane	0 mm	0 mm	0 mm	26.7 mm	-
	Ethyl acetate	10.7 mm	8.5 mm	6 mm	22.7 mm	-
C. albicansATCC 10231	N-hexane	0 mm	0 mm	0 mm	27.5 mm	-
	Ethyl acetate	0 mm	0 mm	0 mm	26.9 mm	-

IV. CONCLUSION

The findings of the study may be helpful to future investigators in identifying alternative and new bioactive secondary metabolites like antibiotics to treat resistant human pathogens.

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