



Antiproliferative Activity of Methanol Fractions from the Stem Bark of *Garuga floribunda* Decne Against A549 Cells

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Abstract. The stem bark of *Garuga floribunda* Decne, commonly known as Buhu tree, has traditionally been used for treating tuberculosis, lung diseases, and cancer. It contains cyclic diarylheptanoids, primarily known for their diverse bioactivities such as anticancer, antioxidant, and anti-inflammatory properties. The research was undertaken due to the lack of prior studies on the anti-cancer potential of Buhu stem bark. This study aims to evaluate methanol extract from Buhu stem bark on A549 cells using the MTT assay. The study evaluated the toxicity of methanol extract obtained through maceration using the Brine Shrimp Lethality Test (BSLT) and its potential as an anticancer agent against A549 cancer cells using the MTT method. The methanol extract from Buhu stem bark confirmed the presence of tannins, saponins, flavonoids, alkaloids, and terpenoids. The results indicated that the methanol extract, tested using the BSLT method, exhibited toxicity with an LC₅₀ value of 60.04 ppm. Furthermore, it was determined that it had antiproliferative activity. The methanol extract from Buhu stem bark showed diverse cell inhibition percentages at different extract concentrations. The methanol extract exhibited moderate cytotoxic activity with an IC₅₀ value of 768.117 ppm. It is considered a potential anticancer agent.

Keywords: Antiproliferative Activity, mGaruga floribunda, A549 Cells

1 Introduction

The Buhu plant, commonly known as Garuga, belongs to the Burseraceae family and is classified as a tree species [1]. The plant is frequently encountered in the Southeast Asian region, Australia, and the Western Pacific, exhibiting robust growth at elevations and sea levels of around 400 meters [2, 3]. *Garuga floribunda* Decne has been recognized as a traditional medicinal plant by the Gorontalo community, known locally as 'Buhu'. Traditional medicinal preparations consist of a mixture of natural ingredients processed using straightforward methods, resulting in specific therapeutic effects. Additionally, knowledge about the use of the medicine is often passed down from generation to generation within the community [4, 5]. Herbal medicine consists of plants containing one or more active ingredients believed to have medical benefits and is utilized in traditional medical practices [6].

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Parts of the *Garuga floribunda* Decne plant, such as the sap from its bark, have been used as antibacterial drugs to treat infections caused by insects. The roots of this plant are also used in the treatment of lung infections, while the bark is often used in postpartum care for women” [7]. Leaf extract can be used as an anti-malarial [8, 9]. Acetone extract of *Garuga floribunda* Decne bark is active against Murine leukemia P-388 cells [10].

The stem bark of the Buhu plant is often used in traditional medicine because it contains various active compounds such as alkaloids, arbutin, fats and oils, saponins, tannins, and organic acids such as oxalic, formic, and tartaric acids. The presence of various active compounds results in a variety of biological activities, giving the bark of the Buhu plant benefits for various health conditions [2]. This plant is known to contain major secondary metabolites in the form of cyclic diarylheptanoid groups, which have various biological activities, including anticancer, antibacterial, antioxidant, antidiabetic, and anti-inflammatory properties [11, 12]. Chemical compounds found in this plant can be further identified and studied for potential development as alternative materials in natural-based medicine [13].

Natural compounds are considered a source of bioactive compounds that have therapeutic potential. In recent decades, great efforts have been made to discover new natural products from microbes, plants, and other organisms, aiming to test the ability of natural compounds to fight cancer and understand how they work [13, 14]. Many diverse active physiological chemical compounds are naturally found. Currently, most chemotherapy agents are derived from natural sources, and the use of natural products in cancer treatment is on the rise. According to a study by Newman et al. (1981-2014), many anticancer drugs are obtained from nature. Of the 174 approved anticancer drugs, 77% are classified as semi-synthetic drugs and natural compounds [15, 16].

A literature search shows that research on the anticancer activity of Buhu stem bark is still limited. In this study, fractionation of the stem bark of *Garuga floribunda* Decne was carried out using methanol. The methanol extract was then tested for anticancer bioactivity using the MTT assay method on A549 cancer cells.

2 Method

2.1 Materials

The sample used in this study was the bark of Buhu (*Garuga floribunda* Decne) obtained from Daenaa village, West Limboto District, Gorontalo Regency, Gorontalo Province. Determination was carried out at the Indonesian Biology Generation Laboratory, Gresik, East Java. The result of the determination confirmed the plant as named “*Garuga floribunda* Decne” in Latin. This plant belongs to the Burseraceae family. The sample was then ground and dried in the air without direct exposure to sunlight.

2.2 Preparation and Extraction of Buhu Stem Bark

The sample was weighed, with each sample being 1 kg. Maceration was carried out using methanol for 3 x 24 hours. The extract obtained was concentrated using a rotary evaporator at a temperature of 40°C until a thick extract was obtained and no longer had a methanol smell.

2.3 Brine Shrimp Lethality Test (BSLT)

Larvae of *Artemia salina* Leach are obtained by hatching *A. salina* eggs that have been incubated in seawater for 48 hours. Ten larvae are placed in a 1.5 mL eppendorf tube and then added with extract that has been dissolved in seawater, with concentrations of 21.25, 62.5, 125, 250, 500, 1000, and 2000 ppm and incubated for 24 hours under light. As a control, 10 *A. salina* Leach larvae are used without extract. Significant mortality of shrimp larvae indicates the presence of a strong cytotoxic component in a plant. The number of shrimp larvae deaths is recorded and then the percentage of their mortality is calculated.

2.4 Antiproliferative Activity

Cells of cancer grown in T25 flasks were subcultured, then the cells were cultured in a 96-well tissue culture plate with a total of 5000 cells/well and then incubated in growth medium at a temperature of 37°C and 5% CO₂ for 24 hours. Each methanol fraction concentration was added 100 µl/well, cells that were not treated were included as control cells, which were then incubated for another 48 hours. The compound 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added and incubated at a temperature of 37°C and 5% CO₂ for 4 hours. supernatant cells were discarded, the formazan crystals formed were dissolved in 70% ethanol; Absorbance measurement was performed using a microplate reader at a wavelength of 565 nm.

2.5 Data Analysis

50 µl of cell solution was added. 50 µl of trypan blue was flowed into the hemocytometer, then live cells were observed and counted from 2 large squares (no color absorption). The results obtained were then calculated with equation 1.

$$\text{Number of cells per mL} = \frac{n}{4} \times 10^4, \quad (1)$$

where n is number of cells in the entire chamber.

3 Result and Discussion

The process of extracting chemical components from stem bark of *Buhu* is carried out by maceration method. The methanol extract yield obtained was 5.4%. The purpose of phytochemical screening of methanol extract is to identify the content of chemical compounds in the sample. Phytochemical screening includes tests for flavonoids, alkaloids, terpenoids, steroids, tannins, and saponins.

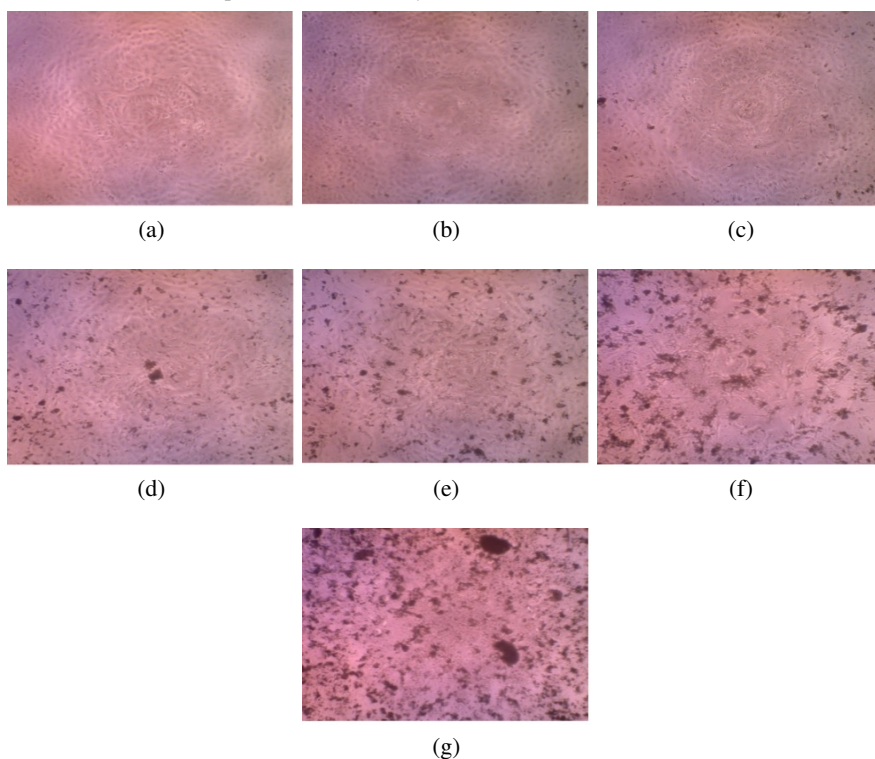


Fig. 1. Morphology of A549 cells due to MTT treatment

Table 1. The results of phytochemical screening of methanol extract from stem bark of Buhu

Phytochemical screening	Reagent	The results
Flavonoid	HCl + Mg	(yellow) (+) flavonoid
	H ₂ SO ₄	(dark green) (+) flavonoid
	NaOH	(Yellow) (+) flavonoid
Alkaloid	Dragendorff	(Dark yellow-brown precipitate) (+) alkaloid
	Wagner	(Orange-orange Precipitate) (+) alkaloid
	Mayer	(brown-brown precipitate) (+) alkaloid
	Hager	(Brown-no precipitate) (-) alkaloid
Triterpenoid	Anhydride acetic + H ₂ SO ₄	(red ring) (+) terpenoid
Tanin	FeCl ₃	(blackish-green) (+) tanin
Saponin	Hot Aquadest	(foam formed) (+) saponin

Table 2. The BSLT Test Results of the Stem Bark of Buhu

Fraction	Concentration (ppm)	% Mortality of Cell
Methanol	31.25	36.67
	6.25	53.33
	125	66.67
	250	76.67
	500	86.67
	1000	96.67
	2000	100
Ethyl acetate	31.25	16.67
	62.50	30.00
	125	56.67
	250	76.67
	500	90
	1000	96.67
	2000	100

Table 3. LC50 Values of Methanol and Ethyl Acetate Fractions from Buhu Stem Bark Determined Using the BSLT Method

Sample (Fraction)	LC50 (ppm)
Methanol	60.10

Table 4. Phytochemical Test of Isolate 2N

Reagent	Result	Compound
Chloroform, Acetic Anhydride, H ₂ SO ₄	Formed a Red Ring	(+) Terpenoid

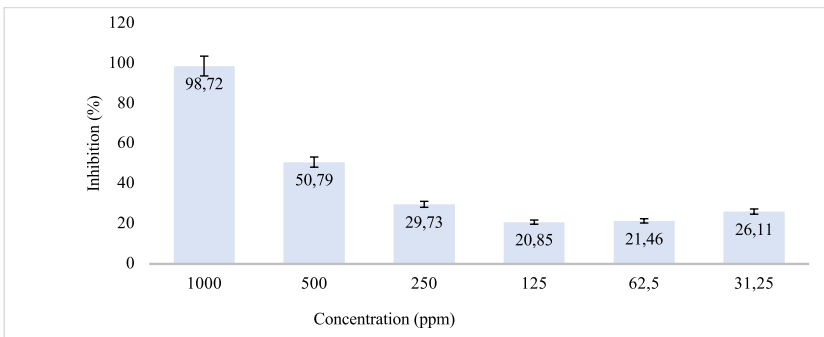


Fig. 2. Antiproliferative activities of methanol extract of Buhu stem bark

4 Conclusion

The stem bark extract of buhu (*Garuga floribunda* Decne) obtained has a yield percentage of 5.4%. The qualitative phytochemical test results of the methanol extract of the

stem bark of buhu (*Garuga floribunda* Decne) are positive for flavonoids, alkaloids, terpenoids, tannins, and saponins. The methanol extract of the stem bark of buhu (*Garuga floribunda* Decne) has antiproliferative activity with an IC₅₀ value of 768.117 $\mu\text{g/mL}$. The test results fall under the moderate category.

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