

RESEARCH ARTICLE

Halal Critical Point Analysis of *Bajakah* Wood (*Spatholobus littoralis* Hassk.) Nano Particle as Anticancer Agent

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Abstract

Bajakah wood contains phenolic compounds, flavonoids, tannins, and saponins with anticancer activity. The discovery and development of new drugs require several stages. In the process, there are many possibilities of adding other substances to form new active substances or as solvents that allow drug preparations to be doubtful of halalness. Hence, it is necessary to analyze the critical point of halal ingredients. The purpose of this study was to determine the characteristics of the nanoparticles of *bajakah* wood (*Spatholobus littoralis* Hassk.) and to test the anticancer activity in several cancer cell cultures, as well as to analyze the critical point of halalness of the material. This research method is a composition test using chromatography and anticancer activity test using MTT. Analysis of the critical point of halal materials using hazard analysis critical control point (HACCP). The research was carried out at the Indonesian Engineering Nanotechnology Laboratory South Tangerang and the UGM Integrated Laboratory Sleman in July–December 2020. The results showed that *bajakah* wood nanoparticles contained pure water as a solvent, viscosity 0.08878 cP, scattering intensity 1.1059 cps, diameter 176.1+/-43.7 (nm). Cytotoxic test results showed IC₅₀ against cell culture MCF7 1,063.28 (±114.98) g/mL, HepG2 53.34 (±0.35) g/mL, T47D 150.63 (±8.44) g/mL, WiDR 114.38 (±7.82) µg/mL, HTB 97.50 (±3.49) µg/mL, HeLa 182.95 (±36.22) µg/mL, and Vero 710.10 (±106.46) µg/mL. This study concludes that *bajakah* wood nanoparticles are not critical in terms of halal ingredients. At the same time, their anticancer activity is weak against breast cancer and uterine cervical cancer, medium categories against liver cancer and lung cancer, and is not toxic to normal cells.

Keywords: Anticancer, *bajakah* wood, critical halal point, nanoparticle

Analisis Titik Kritis Kehalalan Nanopartikel Kayu Bajakah (*Spatholobus littoralis* Hassk.) sebagai Agen Antikanker

Abstrak

Kayu bajakah mengandung senyawa fenolik, flavonoid, tanin, dan saponin yang memiliki aktivitas antikanker. Penemuan dan pengembangan obat baru memerlukan beberapa tahapan. Dalam prosesnya terdapat banyak kemungkinan penambahan zat lain untuk membentuk zat aktif baru atau sebagai pelarut yang memungkinkan sediaan obat diragukan kehalalannya. Oleh sebab itu, perlu dilakukan analisis titik kritis kehalalan bahan. Tujuan penelitian ini mengetahui karakteristik sediaan nanopartikel kayu bajakah (*Spatholobus littoralis* Hassk.) dan menguji aktivitas antikanker pada beberapa kultur sel kanker, serta menganalisis titik kritis kehalalan bahan. Metode penelitian ini adalah uji komposisi menggunakan kromatografi dan uji aktivitas antikanker menggunakan MTT. Analisis titik kritis kehalalan bahan menggunakan *hazard analysis critical control point* (HACCP). Penelitian dilakukan di Laboratorium Nanovasi Rekayasa Indonesia Tangerang Selatan dan Laboratorium Terpadu UGM Sleman pada Juli–Desember 2020. Hasil penelitian menunjukkan sediaan nanopartikel kayu bajakah mengandung pelarut air murni, viskositas 0,08878 cP, *scattering intensity* 1,1059 cps, berdiameter 176,1+/-43,7 (nm). Hasil uji sitotoksik menunjukkan IC₅₀ terhadap kultur sel MCF7 1.063,28 (±114,98) µg/mL, HepG2 53,34 (±0,35) µg/mL, T47D 150,63 (±8,44) µg/mL, WiDR 114,38 (±7,82) µg/mL, HTB 97,50 (±3,49) µg/mL, HeLa 182,95 (±36,22) µg/mL, dan Vero 710,10 (±106,46) µg/mL. Kesimpulan penelitian ini bahwa nanopartikel kayu bajakah bersifat tidak kritis dalam kehalalan bahan. Selain itu, aktivitas antikankernya lemah terhadap kanker payudara dan kanker serviks uteri, sedang terhadap kanker hati dan kanker paru, serta tidak toksik pada sel normal.

Kata kunci: Antikanker, kayu bajakah, nanopartikel, titik kritis kehalalan

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Introduction

Cancer is the leading cause of death globally today. In the 21st-century, cancer is considered the most critical barrier to increasing life expectancy in every country globally.¹ Cancer is a progressive degenerative disease characterized by sequential changes in gene expression mediated by genetic and epigenetic changes in the developing tumor cells. The intrinsic complexity and heterogeneous nature of cancer are often exacerbated by an invasive and metastatic progression which is an obstacle to developing effective cancer therapy.^{2,3}

To date, the efficacy of chemotherapy, which is an important therapeutic modality for cancer, has not increased because it has side effects and high toxicity, and there is treatment resistance. Therefore, anticancer drugs continue to be developed to obtain a more sensitive and specific treatment to use targeted therapy.⁴ The need for new effective drugs for cancer is a commercial opportunity.³ Efforts to find new anticancer agents are to explore natural ingredients, especially medicinal plants. Indonesia's biodiversity that has the potential as traditional medicine is *bajakah* wood or *bajakah tampala* (*Spatholobus littoralis* Hassk.). Based on the preliminary tests carried out, there were positive signs in the phenolic, flavonoid, tannin, and saponin tests.⁵

The process of drug discovery and development requires several stages, starting from the research process at the preclinical and clinical stages and forming drug preparations. This process involves many stages that allow the process of adding other substances to form new active substances or as a solvent available, allowing the drug preparation to become illegitimate. The reference for Muslims to consume halal products is written in the Quran. There are verses in the Quran that call on Muslims to seek *halal* and *toyibban* in their life. Halal means permissible based on the Quran. *Toyibban* means good, clean, hygienic, and healthy.⁶

Halal food is clean and safe without a trace of impurities and is free from prohibited substances (haram), legally enforced by Islamic law. Muslims must have halal food according to Islamic law with healthy ingredients. The phrase "halal food according to Islamic law or halal food" has a broad meaning. First, the food source is obtained legally and free from theft or corruption—that is ethically halal. Secondly, it must be halal in terms of the type or category of things to

be consumed, such as animals or ingredients contained in any food that Islam approves. That is the physical consideration they allow. Apart from the two conditions above, it also focuses on "processing" or the manufacturing stages starting from slaughtering, washing and cleaning, packaging, storage, transportation, selling, and others. Any practices relating to personnel and premises ensure safety, good manufacturing practices, storage, and distribution. Measures are implemented in the food production chain to ensure that food preparation steps are safe for consumption.^{7,8}

Even though the halal industry is receiving much attention nowadays, it still requires many thorough studies. Until now, halal is still often seen as a problem that only involves fiqh scholars. Indeed, the problem of halal and haram is one of the fundamental laws/concepts in Islam. For some people who do not speak Arabic, halal is usually associated with various foods permitted to be taken under Islamic law. Apart from food and beverages, the concept of halal covers a much more comprehensive range of meanings, including behavior, actions, speech, attitudes, clothes, cosmetics, and medicines.⁹ The growing religious awareness of consumers has certainly encouraged producers or the industry to convince consumers by providing products. The halal is labeled as a point of sale or branding.¹⁰ Thus, the halal label stamped on the product is one factor that attracts Muslim consumers in making purchase decisions.

One of the product control methods is the hazard analysis critical control point (HACCP). The HACCP is a scientific, rational, and systematic preventive food safety control and supervision management system to identifying, monitoring, and controlling hazards starting from raw materials. It is conducted during the production/processing, manufacturing, handling, and use of foodstuffs to guarantee that the food material is safe when consumed. The HACCP system is defined as a management to ensure the safety of food products in the food processing industry using the concept of a logical (rational), systematic, continuous, and comprehensive (comprehensive) approach. It aims to identify, monitor, and control hazards with high risk to the quality and safe food products.¹¹ The HACCP concept is rational because the approach is based on historical data about the causes of an illness (illness) and food damage (spoilage). The HACCP is systematic

because the HACCP concept is a detailed and thorough plan and includes operational activities step by step, procedures (procedures), and the size of the control criteria.^{11–13}

The purpose of this study was to determine the characteristics of the nanoparticles of *bajakah* wood (*Spatholobus littoralis* Hassk.) and to test the anticancer activity in several cancer cell cultures, as well as to analyze the critical point of halalness of the material.

Methods

This study was a pure in vitro experimental study, with a randomized post-test only control group design. Random sampling to be included as the experimental and control groups. The allocation of research samples to the experimental group was carried out through random allocation. The research was carried out at the Indonesian Engineering Nanotechnology Laboratory and the UGM Integrated Laboratory in July–December 2020.

The subjects of this study were HTB 183 lung cancer cell culture, MCF-7 breast cancer cell culture, T47D breast cancer culture, WiDr colon cancer cell culture. In addition, we also study HeLa cervix cancer culture, HepG2 liver cancer cell culture, normal kidney cell culture. The test conducted in this study was a nanoparticle preparation of *bajakah tampala* (*Spatholobulus littoralis* Hask) from East Kalimantan, Indonesia.

Simplicia using mechanical milling provided that the milling machine is on for 15 seconds and stops for 2 minutes. It is done 40 times. Sieves the milling results using a 14 mesh (1.41 mm) sieve. Performs milling back to the sieve using wet milling with the addition of 200 mL of distilled water for every 100 grams of the sieve with the condition that it is on for 2 minutes and stops for 3 minutes 90 times. Filter the wet milling results using a 70 mesh sieve, and the nanoparticles were measured using a particle size analyzer.

The characteristic test of nanoparticles of *bajakah* wood was conducted using Beckman Coulter's particle size analyzer. The IC₅₀ is seen by the tetrazolium 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. IC₅₀ is a concentration that can kill 50% of cancer cells. IC₅₀ was calculated using Probit software. The number of cancer cells was counted using a counting chamber with the dilution method. Analysis of the critical point of product halalness

is carried out by tracing the preparation process and testing the alcohol content of the preparation.

After incubation, the culture media was discarded and washed with phosphate-buffered saline (PBS), and 100 µL of MTT solution (1 mL MTT in 10 mL, culture medium) was added to each well and then incubated again at 37°C 5% CO₂ for 4 hours. After 4 hours, 100 µL SDS stopper 10% in 0.1 N HCl was added to each well (to dissolve purple formazan). Then it is stirred for 5 minutes and wrapped tightly, and left at room temperature overnight. After one night at room temperature, the absorption reading was carried out with an ELISA microplate reader at a wavelength (λ) of 595 nm.

From the results of the absorbance analysis, cell viability can be calculated using the formula life (viability)=(cb)/(ab) × 100. Letter a is the absorbance of control cells; b is the absorbance of the media control, and c is the absorbance of the sample. The cytotoxic activity of each of the doxorubicin and cisplatin test compounds was expressed by the IC₅₀ value, which can inhibit cell growth by up to 50% calculated by probit analysis based on the relationship between levels of the inhibitor of cell growth.

This study was approved by the Health Research Ethics Committee, Faculty of Medicine Universitas Islam Bandung, with approval number 388/Komite Etik.FK/VIII/2019.

Results

Bajakah wood is obtained from East Kalimantan, with permission from the head of the Local Office, as much as 10 kg of *bajakah* wood. The next step is to determine which *bajakah* wood will be used to determine the plant species used as the test material—taxonomy of *bajakah* plants presented in Table 1.¹⁴

Bajakah tampala wood preparations of simplicia are dried through winds at room temperature for seven days. After the simplicia

Table 1 Taxonomy of *Bajakah*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Fabales
Genus	<i>Spatholobus</i> Hassk.

Table 2 Characteristics of Nanoparticles of Bajakah Wood

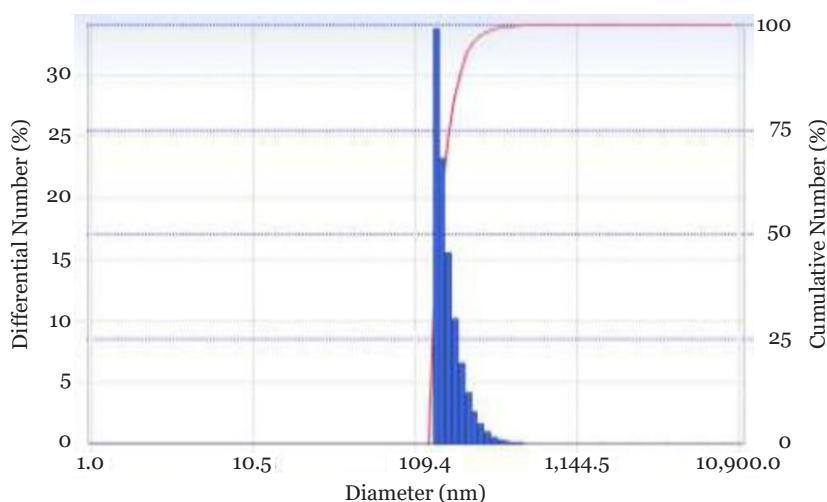
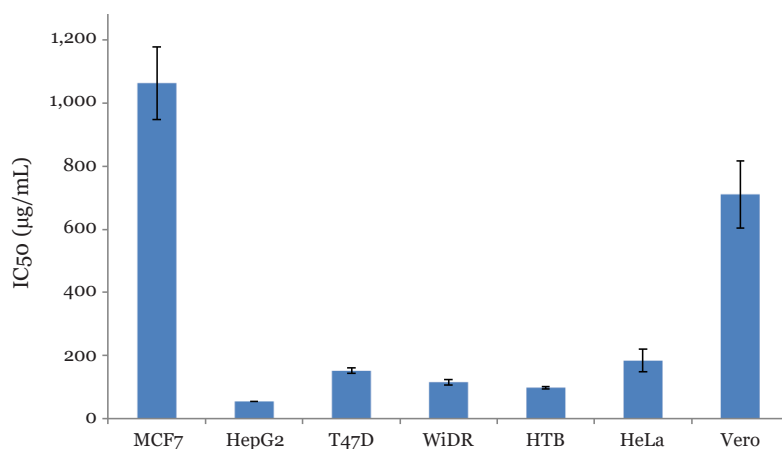
Characteristics	Description
Temperature	25°C
Solvent	Aquadest
Reflative index	1.3328
Viscosity	0.8878 cP
Scattering intensity	11059 cps
Diameter average	176.1+/-43.7 (nm)

is dry, it is chopped so that the simplicia size is smaller and easy to do milling. The process of making nanoparticles is carried out in two stages.

The first step is processing by dry mechanical milling for 15 seconds, 40 times the reinforcement; then, the results are filtered using 14 mesh. Next, the results are carried out wet milling with 200 mL for every 100 grams of the sieve result. The wet milling process is carried out for 2 minutes because there are 90 repetitions.

After wet milling, nanoparticles' nano size and characteristics were carried out using a particle size analyzer. The characteristics of *bajakah* wood nanoparticles are shown in Figure 1 and Table 2.

Measurement of the cytotoxic test of *bajakah* wood nanoparticle preparations was carried out to determine its anticancer potential against various cancer cultures. The test was carried out using

**Figure 1 Characteristics of Nanoparticles of Bajakah Wood****Figure 2 IC₅₀ Bar Chart Graph of Nanoparticle of Bajakah Wood in Various Cancer Cell Cultures**

the MTT method to obtain the concentration of compounds that could inhibit 50% of the tumor cell population (IC₅₀). Tests were carried out at a serial concentration of 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/mL. Cytotoxic test results for each compound are presented in Figure 2.

Figure 2 shows preparation I has IC₅₀ in MCF7 cell culture of 1,063.28 (±114.98) µg/mL, IC₅₀ in HepG2 cell culture 53.34 (±0.35) µg/mL, IC₅₀ in T47D cell culture 150.63 (±8.44) µg/mL, IC₅₀ for WiDR cell culture 114.38 (±7.82) µg/mL, IC₅₀ for HTB cell culture 97.50 (±3.49) µg/mL, IC₅₀ in HeLa cell culture 182.95 (±36.22) µg/mL, and IC₅₀ in Vero cell culture 710.10 (±106.46) µg/mL.

Discussion

Bajakah wood, including traditional medicinal plants that inland communities of Central Kalimantan province, have empirically been used to heal some diseases. Based on previous research, this plant also contains bioactive compounds such as phenolics, flavonoids, tannins, and saponins.^{15,16}

This research generally consists of 3 stages. The first stage aims to determine the characteristics of the *bajakah* wood nanoparticle preparations, where the test results show that there is only water or aqua dest in this preparation. The particle size in this preparation is 176.1+/-43.7 (nm), where the maximum size is below 250 nm, which indicates that this preparation particle is already a nanoparticle category. Nanomaterials, especially nanoparticles, are a vital component in the development of new technologies. Nanoparticle-based technology has developed in various fields, from cosmetics, medical care, medical rehabilitation, the environment to electronic technology. Nanoparticle-based materials change their physical properties with changing particle size. This phenomenon is known as the quantum confinement effect.^{17,18}

In the research and development of drugs with nanoparticle preparations, or so-called nanomedicine, it is thought that they can target drugs at specific receptors. The formulation method can increase the bioavailability of herbal medicines with poor water output. The formation of nano-sized particles can coal drugs and permeability, regulate nanoparticle carriers' physicochemical properties, and increase drug absorption, distribution, and elimination in cells.¹⁹

The second stage of this research is to analyze the critical point of the halal nano-*bajakah* wood preparations. Some preparations often require alcohol as a suitable solvent, but alcohol is a substance or substance included in non-halal or haram. Islam has clear rules regarding the halalness of a product, including medicines. One form of the devotion of a Muslim is consuming halal and good products (*thayibah*).²⁰ The increasing awareness of Muslims regarding the use of halal products is a great potential and opportunity for developing halal drugs and cosmetics. As the world's Islamic cooperation organization, the Organization of Islamic Cooperation (OIC) predicts that these opportunities will continue to grow in the future. This statement is also supported by an increase in the market share of halal medicines and health products in Indonesia.²¹ One of the many herbal medicines developed in Indonesia is an anticancer drug.

Stage 3 aims to see the anticancer effect of *bajakah* wood preparations on various cancer cell cultures. In developing new anticancer drugs as candidates for cancer therapy agents, preclinical testing is crucial to determine its potential cytotoxic activity. The cytotoxic test is used as an early-stage screening to determine the effect of a natural substance in inhibiting tumor cell growth. A compound is considered to have anticancer properties if it can inhibit 50% of the tumor cell population at a specific concentration. The requirements that must be met for a cytotoxicity test system include that the test system can produce a reproducible dose-response curve with low variability. The response criteria must show a linear relationship with the number of cells, and the information obtained from the dose-response curve must be in line with the effect that appears. One of the commonly used methods for determining cell counts is the MTT method.^{22,23}

In this study, the cytotoxic test results of *bajakah* wood nanoparticle preparations against MCF7 cell culture of 1,063.28 (±114.98) µg/mL were classified as having no anticancer effect. IC₅₀ in HepG2 cell culture 53.34 (±0.35) µg/mL, classified as moderate anticancer, IC₅₀ in T47D cell culture 150.63 (±8.44) µg/mL. IC₅₀ in WiDR cell culture 114.38 (±7.82) µg/mL including weak category, IC₅₀ in HTB cell culture 97.50 (±3.49) µg/mL were included in the medium category. In contrast, IC₅₀ in HeLa cell culture 182.95 (±36.22) µg/mL were included in the

weak category. IC₅₀ in Vero cell culture 710.10 (± 106.46) $\mu\text{g/mL}$, showing *bajakah* wood nano preparations are not toxic to normal cells.^{24,25}

Bajakah tampala contains flavonoids, alkaloids, tannins, and phenolic acids, which have anticancer effects. The critical mechanism of flavonoids is to prevent the metabolic activation of carcinogens through phase I metabolic enzymes (cytochrome P450). It can metabolically activate most of the procarcinogens that can trigger carcinogenesis. These flavonoid compounds work by inhibiting the activity of specific P450 isozymes, such as CYP1A1 and CYP1A2, so they have a protective role against induction of cell damage by carcinogen activation.²⁶

Alkaloids and phenolic acids can inhibit cell proliferation processes, stimulate apoptosis, and inhibit the formation of new blood vessels (angiogenesis).^{26–28} Phenolic acid is a material that has bioactivities such as high antioxidants.²⁹ Alkaloids also can inhibit cell survival, proliferation, invasion, and angiogenesis in human glioma cells. In addition, it can induce HeLa cervical cell death with apoptosis and necrosis.²⁹

Conclusions

This study concludes that pirated wood nanoparticles are not critical in terms of halal ingredients. At the same time, their anticancer activity is weak against breast cancer and uterine cervical cancer, categories against liver cancer and lung cancer, while it is not toxic to normal cells.

Conflict of Interest

There was not a conflict of interest in this article.

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