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# Toxicity of Cisplatin and Herbal Medicine Complexed with Bovine Serum Albumin (BSA) and Folic Acid Nanoparticles as Anticancer Candidates

## (Perbandingan Uji Toksisitas Obat Cisplatin dan Obat Herbal Kombinasi Nanopartikel Bovine Serum Albumin (BSA) dan Asam Folat Sebagai Kandidat Antikanker)

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## ABSTRACT

The prevalence of cancer in Indonesia has increased by 8.8 percent in the last two years (2018 and 2020) in terms of adding new cases and mortality. Because of therefor cancer treatment becomes important in reducing the high number of additional new cases and mortality due to cancer patients. Intensive cancer treatment is by surgery, chemotherapy, radiotherapy and immunotherapy. Chemotherapy drugs used for cancer treatment include herbal plant-based cancer drugs (Cancer Fit) and cisplatin its buy from online shop. This study aims to compare the toxicity of herbal drugs (Cancer fit) with cisplatin, a combination of BSA nanoparticles and folic acid as anticancer candidates. The research method is through the synthesis of nanoparticles with the desolvation method, then characterization is carried out using XRD and FT-IR tests. After that proceed to the toxicity test with the BSLT method. The results obtained from XRD analysis on BSA and folic acid nanoparticles from cisplatin (As-NP-BSA-CP) showed a peak of 34.45 while BSA and folic acid nanoparticles from herbal medicine (As-NP-BSA-Oh) obtained a peak of 22.77. The purpose of XRD analysis was to determine the crystalline characteristics of the synthesized nanoparticles and to analyze the crystalline index. FT-IR analysis on (As-NP-BSA-CP) showed that there are functional groups O-H alcohol, C-H, C-C, NO2 that play a role in the synthesis of nanoparticles. Meanwhile, (As-NP-BSA-Oh) showed that the functional groups were C-H alkene, C-O alcohol, and C-N amine/amide. The toxicity level of As-NP-BSA-CP LC50 is 69.14 ppm while the toxicity level of As-NP-BSA-Oh LC50 is 44.14 ppm. Nanoparticles consisting of a combination of bovine serum albumin (BSA), folic acid and cisplatin drugs or herbal drugs can be used as candidates for anticancer drugs.



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## ABSTRAK

Prevalensi penyakit kanker di Indonesia mengalami peningkatan sebesar 8,8 persen dalam waktu dua tahun terakhir (2018 dan 2020) dalam hal penambahan kasus baru dan angka kematian akibat penyakit kanker. Oleh karena itu pengobatan kanker menjadi hal penting dalam mengurangi tingginya jumlah penambahan kasus baru dan angka kematian (mortalitas) karena penderita penyakit kanker. Pengobatan kanker secara intensif adalah dengan pembedahan, kemoterapi, radioterapi maupun imunoterapi. Obat kemoterapi yang digunakan untuk pengobatan kanker diantaranya adalah obat kanker berbasis tanaman herbal (Cancer Fit) dan obat cisplatin yang dibeli dari toko online. Penelitian ini bertujuan untuk membandingkan toksisitas obat herbal (Cancer Fit) dengan cisplatin kombinasi nanopartikel BSA dan asam folat sebagai kandidat antikanker. Metode penelitian melalui sintesis nanopartikel dengan metode desolvasi, kemudian dilakukan karakterisasi dengan uji XRD dan FT-IR. Setelah itu dilanjutkan ke uji toksisitas dengan metode BSLT. Hasil penelitian yang didapatkan dari analisis XRD pada nanopartikel BSA dan asam folat dari obat cisplatin (As-NP-BSA-CP) menunjukkan adanya peak 34,45 sedangkan pada nanopartikel BSA dan asam folat dari obat herbal (As-NP-BSA-Oh) diperoleh peak 22,77. Tujuan dari analisis XRD adalah untuk mengetahui karakteristik kristal dari nanopartikel yang disintesis dan untuk menganalisis indeks kristal. Analisis FT-IR pada (As-NP-BSA-CP) menunjukkan bahwa ada gugus fungsi O-H alkohol, C-H, C-C, $NO_2$ yang berperan dalam sintesis nanopartikel. Sedangkan pada (As-NP-BSA-Oh) menunjukkan bahwa adanya gugus fungsi C-H alkena, C-O alkohol, dan C-N amina/amida. Kadar toksisitas As-NP-BSA-CP $LC_{50}$ 69.14 ppm sedangkan kadar toksisitas As-NP-BSA-Oh $LC_{50}$ 44,14 ppm. Nanopartikel yang terdiri dari kombinasi bovine serum albumin (BSA), asam folat dan obat cisplatin atau obat herbal dapat dijadikan sebagai kandidat obat antikanker.


Kata kunci: Antikanker, Toksisitas, Obat herbal, Obat Cisplatin, Nanopartikel

## INTRODUCTION

Cancer is the most terrible non-communicable disease worldwide in terms of morbidity and mortality (Rahmani *et al.,* 2014). Cancer mortality which continues to increase every year is one of the biggest challenges for the world's health worldwide. Based on data from the Ministry of Health of the Republic of Indonesia in 2018 there were 18.1 million new cases with a death rate of 9.6 million cases of death due to cancer ( Ministry of Health RI Agency for Research and Development, 2018).

Treatment of cancer has been carried out intensively, namely by surgery, chemotherapy, radiotherapy, and immunotherapy. This treatment (surgery, chemotherapy, radiotherapy and immunotherapy) is effective for killing cancer cells but has weaknesses, namely causing non-specific drug targets, increasing multi-drug resistance (MDR), and can affect normal cells for cancer patients. This gives rise to normal cell tissue damage and cause serious side effects on the patient. Chemotherapy is one solution to the MDR phenomenon (Mutiah *et al.,* 2018).

One of the chemotherapy drugs used for cancer treatment is cisplatin. Cisplatin, or cis-diaminedichloroplatinine (II) is the first compound in the planar rectangular platinum (II) series to contain chemotherapy drugs, including Carboplatin and Oxaliplatin. The platinum complex reacts in the body by binding to DNA causing the DNA strands to crosslink which triggers the cells to die. Cisplatin works as an anti-cancer by using DNA cross-links and performs apoptosis in healthy cells and cancer cells themselves. Cisplatin is given intravenously as a short-term infusion in saline solution for the

treatment of malignant cancer. The clinical use of cisplatin is limited because of its dose-dependent toxic side effects. Because the resulting side effects can reduce the quality of life of cancer patients (Qi *et al.,* 2019). In addition to using the drug cisplatin, cancer treatment can also be done using herbal medicines. Cancer treatment from herbal plants continues to advance in the clinical field. Herbal medicine is an alternative solution to reduce toxicity and normal cell damage for cancer patients. Effective cancer treatment is based on a combination therapy approach, namely by using herbal medicines (Cancer Fit) or a combination of chemical drugs to minimize complications (Parhi *et al.,* 2012).

BSA nanoparticles derived from albumin protein have properties such as selectivity in drug delivery, biodegradation, non-toxic, safe, easy to purify, and can dissolve in water. While folic acid is a natural ligand in vitamin (B9) it is important for cell proliferation, DNA biosynthesis, and metabolism and can be used in cancer therapy. This research analyzes herbal medicine and cisplatin drugs combined with bovine serum albumin (BSA) and folic acid nanoparticles to reduce drug toxicity, drug delivery, biodegradation, bioavailability, and drug solubility. The steps carried out in this research are a synthesis of cisplatin drug formulations and herbal cancer drugs with a combination of bovine serum albumin (BSA) and folic acid nanoparticles. Toxicity test using the BSLT (Brine Shrimp Lethality Test) method, so it can be used as an anticancer candidate.

## MATERIAL AND METHODS

### Materials

This research is experimental and carried out at the health chemistry laboratory, Nahdlatul Ulama Surabaya of university. Materials used in the study: The results of the synthesis of BSA nanoparticles with cisplatin (CP-NP-BSA), folic acid nanoparticles with BSA-cisplatin (As-NP-BSA-CP) nanoparticles, for the synthesis method according to the journal (Nidianti, Andini, *et al.,* 2020). The results of the synthesis of BSA nanoparticles with herbal medicine (NP-BSA-Oh), folic acid nanoparticles with BSA nanoparticles - herbal medicine (Cancer Fit), and folic acid (As-NP-BSA-Oh). For the synthesis method according to the journal (Nidianti, Wulan, *et al.,* 2020). NaOH solution merck, biuret reagent muda berkah, PBS solution Ph 6, Gluteraldehyde and sorbitol.

### Methods

**Synthesis of NP-BSA with Cisplatin Drug (CP-NP-BSA)**

The NP-BSA formed was added with injection cisplatin comersial of 10 mg incubated for 24 hours, 37 °C then stirred at 600 rpm then added 5 mL of 0.1 N NaOH. The results of the centrifugation at 2000 rpm for 10 minutes formed were then dried using a hotplate and oven at a temperature of 40 °C for 6-8 hours to obtain a powder form (Nidianti, Andini, *et al.,* 2020).

**Synthesis of CP-NP-BSA Combination of Folic Acid (As-NP-BSA-CP)**

CP-NP-BSA was modified with folic acid. Folic acid was added with PBS and Sorbitol in a ratio of 1:2:2 then stirred for 15 minutes at room temperature (28-32 $^{o}$C). The amount of CP-NP-BSA added was 20 mg/mL each and stirred with a magnetic stirrer for 4-5 hours. The results of the centrifugation formed were then dried using a hotplate and oven at 40 $^{o}$C for 6-8 hours to obtain a powder form (Nidianti, Andini, *et al.,* 2020).

**Synthesis of Bovine Serum Albumin (BSA) Nanoparticles - Cancer herbal medicine (NP-BSA-Oh)**

The method of making BSA nanoparticles with cancer herbal medicine was carried out by adding 0.2 grams of BSA into 3.2 ml double distilled and stirring 600 rpm at room temperature for 15 minutes. Then add 6.4 ml of herbal solution (12 mg of herbal medicine (cancer fit) in 1 ml of acetone is desolvation). Added dropwise at room temperature and in the dark. Added 4 mg of ascorbic acid is reductor and stirred for 2 hours. Then centrifuged at 18,000 rpm for 15 minutes 3 times and washed with double distilled and then dried overnight (Nidianti, Wulan, *et al.,* 2020).

**Folic Acid Synthesis with BSA Nanoparticles – Cancer herbal medicine (As-NP-BSA-Oh)**

BSA nanoparticles - herbal medicine from the previous procedure. Then conjugated with folic acid on the surface of BSA nanoparticles was carried out by adding folic acid (27 mg), ascorbic acid (58.6 mg), and ascorbic acid solution (35.2 mg) with DMSO added as a solvent. The solution was reacted by stirring (stirrer) for 30 minutes and protected using aluminum foil. The folic acid solution was added by adding dropwise into the solution of BSA nanoparticles – herbal medicine up to a pH of 8.5. To reacted, stored overnight and stirred at room temperature. Purification was carried out using 20,000 rpm centrifugation for 10 minutes, to remove unconjugated folic acid. Then the drying process is carried out on the product (Nidianti, Wulan, *et al.,* 2020).

**Characterization using XRD**

The sample (NP-BSA, As-NP-BSA-CP, NP-BSA-Oh and As-NP-BSA-Oh) to be analyzed is inserted into an aluminum plate measuring 2x2 cm, the aluminum plate containing the sample is characterized using XRD with a Cu-Ka1 source setting the wavelength and diffraction angle of 2θ. For graph, interpretation use the help of Match software version 3.8.1 (Analysis Kualitatif XRD) (Nidianti, Wulan, *et al.,* 2020).

**Characterization using the FT-IR Spectrophotometer**

The chemical structure of the sample was known through Fourier Transform Infrared (FT-IR) Spectroscopy (Bruker, Tensor 27). Standard disks were collected by mixing 2 mg of the sample with 200 mg of KBr medium and grinding to a fine powder and then compressing the resulting powder into

transparent pellets (pressure, 12 Tons). FT-IR spectra were recorded using a wavelength of 400 to 4000 $cm^{-1}$ (Nidianti, Wulan, *et al.,* 2020).

**Brine Shrimp Lethality Test (BSLT) Method Toxicity Test**

Artemia salina eggs 0.5 g were hatched in seawater (35% salt content), with good air circulation and bright lighting at a temperature of 24-26 $^{o}$C. After 12 - 48 hours the eggs hatch, the salinity is reduced to 5%. For the BSLT test, Artemia salina larvae should not be 7 days old and should not be less than 3 days old. For pure compounds, concentrations of 1, 10, and 100 ppm were made. Prepare 10 vials (test tubes) each filled with 5 ml of seawater and 5 ml of sample solution with varying concentrations and 10 Artemia salina. Do also for control with solvent (ethanol). Contact time is viewed as 1 hour, 6 hours, 12 hours, and 18 hours. Replication should be 3 X, so that the deviation is better, calculate LC50 with SPSS. Because Artemia salina is in direct contact with the sample, it can be seen the number of live and dead Artemia With the following formula (Meyer *et al.,* 1982) :

$$\%\ Mortality = \frac{Total\ larvae\ mortality}{Total\ larvae}\ \text{x}\ 100\%$$

## RESULTS AND DISCUSSION

The synthesis of Bovine Serum Albumin (BSA) nanoparticles with cisplatin/cancer herbal medicine was carried out by the desolvation method (ie, the technique of making nanoparticles based on the difference in solubility between the desolvating agent and water solvent mixed with BSA). The solubility of BSA in water is high when a desolvating agent is added, such as (ethanol, acetone). In this study, a desolvation agent, namely acetone, was used to form an aggregate of BSA.

In this research, is carried out continuously. First, the synthesis of Bovine Serum Albumin (NP-BSA) nanoparticles then the results of the synthesis are continued by adding the drug cisplatin to form (CP-NP-BSA). Then the results of CP-NP-BSA were synthesized with the addition of folic acid to form (As-NP-BSA-CP). The results can be seen in Figure 1 (Nidianti & Andini, 2021).

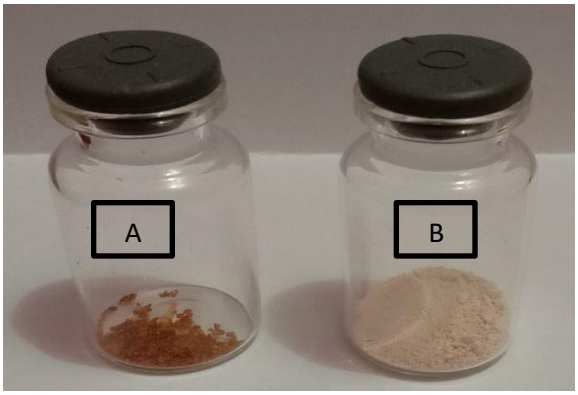


Figure 1. Powder of Cisplatin Conjugated Nanoparticles
A.) NP-BSA, B.) Folic Acid As -NP-BSA-CP

Preparation of herbal medicine BSA nanoparticles (NP-BSA-Oh) was carried out by continuing the procedure for BSA nanoparticles. Herbal medicine was formed and a crosslinker was added, namely folic acid (As-NP-BSA-Oh. The results can be seen in Figure 2 (Nidianti, Wulan, *et al.,* 2020).

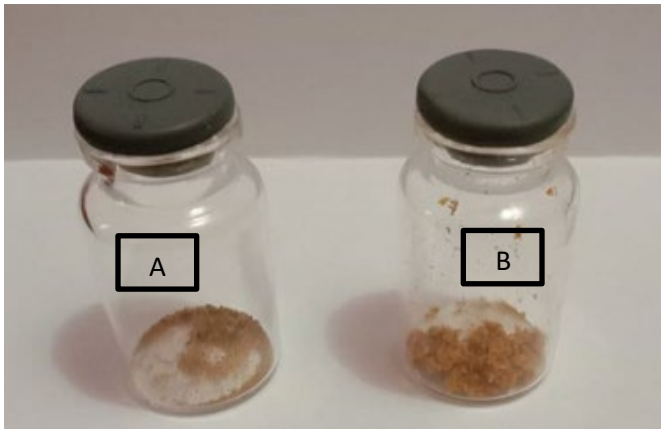


Figure 2. Powder of Herbal Medicines Conjugated Nanoparticles
A.) NP-BSA-Oh B.) As- NP-BSA-Oh

The characterization of NP-BSA nanoparticles and As-NP-BSA-CP nanoparticles was carried out using an XRD instrument. Indexing is defined as the unit cell dimension through the resulting peak positions. XRD analysis in this study used a diffraction angle of 2θ with an angle of 10°-90° for all types of samples (Kumaran *et al.,* 2017). The results of the XRD peak diffractogram on NP-BSA 2θ showed a peak of 31.69°; 45.41°; 56.44°; 66.28° and 75.29°. Meanwhile, the results of the XRD peak diffractogram on As-NP-BSA-CP are 2θ, peaks appear at 22.51° and 34.45°. The results of the diffractogram show a shift in peaks between NP-BSA and As-NP-BSA-CP because the nanoparticle crystals obtained from the synthesis are not pure (Kasim *et al.,* 2020). Differences in peaks of NP-BSA and As-NP-BSA-CP due to different components. In the synthesis of As-NP-BSA-CP, there is the addition of folic acid, cisplatin drug, and glutaraldehyde crosslinker (Nidianti, Andini, *et al.,* 2020). XRD results can be seen in Figures 3 and 4.

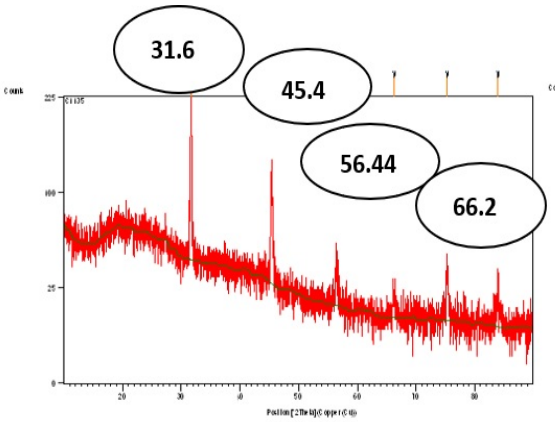


Figure 3. NP-BSA XRD analysis

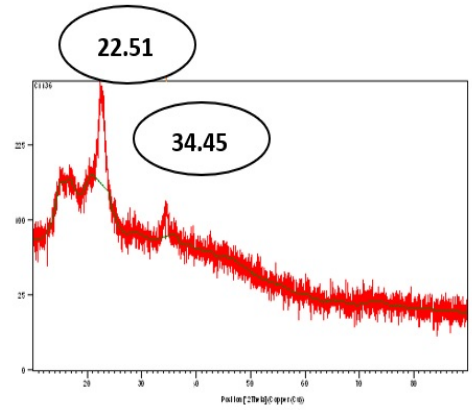


Figure 4. As-NP-BSA-CP XRD analysis

XRD analysis was performed to analyze the crystalline index. The indexer is defined as the determination of the unit cell dimensions through the resulting peak position. In the XRD analysis, this study uses 2θ with an angle of 10°-90° for all types of samples (Abdullah & Khalil, 2017). The results of the XRD peak diffractogram on NP-BSA-Oh appear at 22.27° while the XRD peak diffractogram results at 22.77°. XRD results can be seen in Figures 5 and 6.

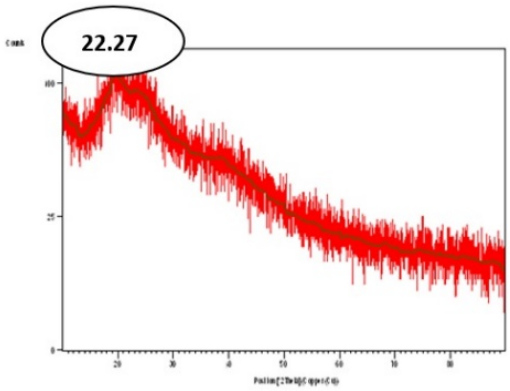


Figure 5. NP-BSA-Oh XRD analysis

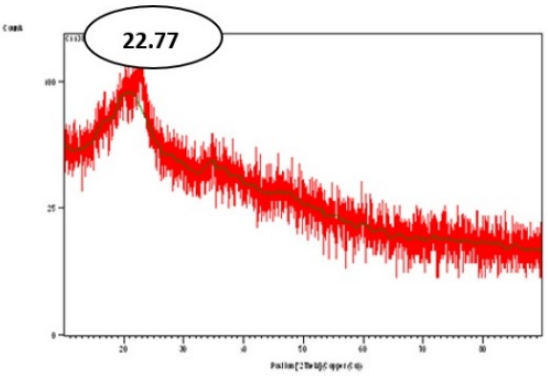


Figure 6. As-NP-BSA-Oh XRD analysis

Table 1. FT-IR Nanoparticle Spectrum Results

| Sample Code | Functional groups | Wave Number ($cm^{-1}$) |
|---|---|---|
| NP-BSA | C-H Alkene | 975 |
| | C-O Alcohol/Eter/Carboxylic acid /Ester | 1107, 1192 |
| | C-N Amine/Amide | 1360 |
| | C=C Alkyne | 2819, 2872 |
| As-NP-BSA-CP | C-H Aromatic | 3169 |
| | C-H Alkene | 671-931 |
| | C-O Alcohol/Eter/Carboxylic acid /Ester | 1041-1317 |
| | NO2 | 1317 dan 1554 |
| | C=C Aromatic ring | 1554 |
| | C=C Alkyne | 2133 |
| | C-H Alkane | 1336-1371 |
| | C-O | 2065 |
| | C-H | 29014 |
| | O-H Alcohol | 3246-3300 |
| NP-BSA-Oh | C-H Alkene | 779 |
| | C-H Alkane | 2873 |
| | O-H Alcoholl/Phenol | 3323 |
| | C-H Alkene | 705 |
| | C-H Alkene | 759 |

| | | |
|---|---|---|
| As-NP-BSA-Oh | C-O Alcohol/Eter/Carboxylic acid /Ester | 1153 |
| | C=C Alkyne | 2860 |
| | C=C Alkyne | 2902 |
| | C=C Alkyne | 2916 |

FT-IR analysis provides information about the functional groups contained in the nanoparticles NP-BSA, As-NP-BSA-CP, NP-BSA-Oh, As-NP-BSA-Oh. The spectrum of the FT-IR results shows the wave number value in the sample, the results can be seen in table 1 (Puspitarum, 2018).

Toxicity can be defined as the ability of a substance to cause damage to living organisms (Millati, 2016). The toxicity test of the BSLT method was carried out using Artemia salina shrimp larvae. The results of the probit analysis using SPSS showed the LC50 value. The toxicity level of As-NP-BSA-CP LC50 is 69.14 ppm while the toxicity level of As-NP-BSA-OH LC50 is 44.14 ppm. The LC50 value of the combination of folic acid nanoparticles with cisplatin was higher than that of herbal drugs.

## CONCLUSION

Based on the results of the research that has been done, it can be concluded that cancer herbal drugs and cisplatin drugs combined with Bovine Serum Albumin and Folic Acid nanoparticles have a low toxicity range and can be used as drug candidates in cancer therapy.

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## CONFLICT OF INTEREST

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