

## The effect of syrup simplex concentration on the physicochemical stability of Gembili's inulin (*Dioscorea esculenta* (Lour.) Burkill) nanosilver colloid

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### ABSTRACT

Gembili (*Dioscorea esculenta*) tuber's inulin is a successful bioreductor agent that forms a nanosilver with a size of 481.4 nm and is stable for 30 days at 4 °C storage. That nanosilver has immunomodulatory activity and is proven safe from the results of acute toxicity tests at a dose of 4 mg/kgBW. However, a drug delivery system is needed to be developed as a supplement product. The syrup was chosen because alcohol free, has a better taste, measured dose, and stable to maintain of the active substance compared to elixir, solution, and suspension. Syrup simplex as a syrup base affects stability because it has the potential to form crystals during storage. This research aims to determine the effect of syrup simplex concentration on the physicochemical stability of nanosilver colloid. The research was conducted with the biosynthesis process using gembili's inulin, nanosilver characteristic, modified syrup formula, and stability test. Nanosilver syrup was prepared by modifying the syrup simplex concentration of 20%, 40%, and 60%. Optimum dose of nanosilver as imunomodulator was added, then tested the physicochemical; stability, including organoleptic, pH, and viscosity, before and after storage with temperature variations of 4 °C and 40 °C for six cycles. The choosen formula then analysis using FT-IR and sugar reduction content. Data analysis using SPSS 21.0 Windows with One-way ANOVA. The results showed that the concentration of syrup simplex affected consistency, pH, and viscosity. The syrup simplex concentration of 60% met the requirement with a medium thick consistency, pH 5.25±0.03, a viscosity of 92±2.6 cps, reducing sugar content was 20.59% ±0.002, and the FTIR profile showed that it still contained nanosilver which was indicated by the presence of Ag-N groups compared to silver nitrate solution.

**Keywords:** nanosilver; syrup simplex, immunostimulant, gembili's inulin, physicochemical stability

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## INTRODUCTION

Nanosilver is a product based on nanotechnology. Currently, nanosilver is developed as a medical agent and has been tested. Silver ions can cause toxicity to the body's tissues because they produce a high affinity for thiols in the liver and other organs (Tiwari et al., 2011). When silver is made into nanoparticles, there is an increase in the contact area with bacteria which causes more effective antibacterial activity and reduces toxicity (Ahmed et al., 2018). In addition to antibacterial activity, nanosilver has antiviral activity (Pulit-Prociak et al., 2015). According to (Harso, 2017), nanosilver is a catalyst for the body's immune system to kill viruses, pathogens, and bacteria in humans.

Biosynthesis of silver nanoparticles using plant extracts is safer for the environment than physical and chemical methods. The polysaccharide group can be a reducing and nanosilver-capping agent. Gembili tubers are local tubers that contain inulin oligosaccharides which can enhance the body's defense mechanism (immunomodulator) (Shafie et al., 2022). Inulin is a carbohydrate that contains fiber from plants and is widely used in the world of health as a source of prebiotics to increase body immunity (Azhar, 2009). Gembili's inulin can be an ion silver bioreductor with immunomodulatory activity. In synergy with this, the nanosilver can increase the solubility of gembili's inulin (Ermawati et al., 2021). Gembili's Inulin, a silver bioreductor, produces particle sizes in the nanometer range (12.4–48.0 nm) with a spherical shape. Intense antibacterial activity against gram-positive and weak against gram-negative bacteria (Ermawati, 2017).

The silver used as medical treatment is silver nitrate ( $\text{AgNO}_3$ ), not silver ion ( $\text{Ag}^+$ ). The toxicity effect of silver nitrate after being made in nanoparticle form is lower, and the effectivity as an antibacterial agent is increased. The maximum concentration of nanosilver in the human body is 10 mg/Kg BW (Pulit-Prociak et al., 2015). So, the concentration of nanosilver used in this research is safe for humans, which is also supported by further research, namely the immunomodulator test and toxicity test.

The immunomodulator test of gembili's inulin nanosilver based on the Immunoglobulin G (IgG) profile on the serum's tested animal, which was induced by the vaccine at a dose of 4 mg/kgBW, with the ELISA reader method had the highest Optical Density value compared to other doses. The comparison with synthetic immunostimulants produced sig.  $0.13 > 0.05$ , which means not significantly different and effective as an immunomodulator with immunostimulating activity. The nanosilver solution was stable for 30 days of testing at an optimum pH of 8 (Nurani, 2022). The results of the acute toxicity test of Gembili's inulin nanosilver showed no toxicity symptoms in the first 4 hours of treatment and 24 hours of observation. They had not yet reached the LD50 value. Observations on the semiquantitative histological profile of the kidneys and liver showed reversible damage in congestion and intratubular bleeding at a dose of 254 mg/kgBW (Setianingsih, 2020).

Based on the facts of previous research, gembili's inulin nanosilver will be developed as a supplement product. The syrup was chosen because alcohol free, has a better taste, measured dose, and stable to maintain of the active substance compared to elixir, solution, and suspension. Simplex syrup as a syrup base affects stability because it has the potential to form crystals during storage. The effect of adding sugar on the characteristics of red dragon fruit syrup with concentration ratios of added sugar of 50, 55, and 65%. Dragon fruit syrup produced by adding 50% sugar has met the SNI (2013) syrup quality standard: a reduced sugar content of 65.7%. According to (Fickri, 2019), the quality requirement in making syrup is the maximum concentration of sugar solution in syrup at room temperature of 65%. If it is higher, crystallization will occur, but if it is lower than 60%, the syrup will be contaminated by microbes. Nanosilver syrup formulation has been carried out before giving nanosilver syrup to honeybees at a dose of 25 ppm, or the equivalent of 25 mg/L nanosilver can improve the health of bees (Grzegorz et al., 2013).

This study will incorporate gembili's inulin nanosilver into syrup preparations. The process of making nanosilver syrup was carried out by adding gembili's inulin of 4 mg/kgBW by modifying the concentration of simplex syrup to 20%, 40%, and 60%, then evaluated on physicochemical properties. This study aims to determine the effect of simplex syrup concentration on the stability of nanosilver

syrup preparations which are expected to accept by patients of various ages for immunomodulatory supplements.

## MATERIALS AND METHOD

### Materials

Gembili tubers, Cepogo, Central Java, Indonesia (*Dioscorea esculenta* (Lour.) Burkill) with document number of 130/UN27.9.6.4/Lab/2022; silver nitrate (AgNO<sub>3</sub>, Merck KgaA, Germany); 30% ethanol (repackaged by Toko Saba Kimia, Surakarta; simplex syrup (repackaged by PT. Agung Jaya Indonesia); propylene glycol (repackaged by PT. Agung Jaya Indonesia); Na benzoate (repackaged by Toko Saba Kimia, Surakarta ); aquadest (Repackaged by PT. Agung Jaya Indonesia), Anhydrous glucose pro analysis Specification 1.08337.11000 (Repackaged by Planet Kimia, Jakarta), Nelson reagent (Repackaged by CV Muda Berkah, Yogyakarta), Arsenomolybdate reagent (Packaged repeated by CV Adi Mandiri, East Java. Instrument: Oven (Mermmet UN110, Germany); pH meter Eutech CyberScan pH 300 (OHAUS Starter 300); Viscometer Rion VT-04F (Japan); UV Visible Spectrophotometer (Genesys 150, USA); FTIR (Shimadzu); Centrifuge (Thermo Scientific, German).

### Methods

#### Gembili's inulin preparation

Determination of gembili tubers was carried out at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia. Gembili tuber flour is dried in an oven at 40 °C and dissolved in hot water for 30 minutes. The volume of the resulting filtrate was measured, then ethanol 30% was added to 30% of the total filtrate volume. The solution was stored in a freezer at -10 °C for 18 hours until a precipitate was obtained. The inulin precipitate is dried using an oven (Fera & Masrikhiyah, 2019).

#### Biosynthesis process and characterization of nanosilver

Gembili inulin of 10 grams was dissolved in 250 mL of distilled water at 40 °C and stirred until dissolved. The solution was filtered using Whatman No.1 paper (solution a). Silver nitrate of 85 mg dissolved in 500 mL distilled water at 40 °C (solution b). Solution a (7.0 mL) and solution b (36.0 mL) were incubated at 60 °C for 15 minutes. The biosynthetic solution was deposited for 24 hours to maximize the biosynthetic process. The solution after biosynthesis process were scanning at the maximum wavelength of SPR range of nanosilver using a UV-Vis spectrophotometer, and aquadest was used as a blanko (Ermawati et al., 2021). The biosynthetic solution was deposited for 24 hours to maximize the biosynthetic process. The biosynthesis results were then read at the maximum wavelength using a UV-Vis spectrophotometer, and aquadest was used as a blanko (Ermawati, 2017). Nanosilver characterization was carried out using a Particle Size Analyzer (PSA) to determine the size distribution and uniformity of the particles. The data obtained from this measurement are Z-average, PI (Polydisperse Index), and potential zeta.

Nanosilver syrup was made by carefully weighing the ingredients (Table 1). In the first stage, Na benzoate was dissolved in half of the propylene glycol. Nanosilver was dissolved with the part propylene glycol until dissolved, added with simple syrup until all the ingredients were dissolved, and added aquadest to a volume of 60 mL (Sugarda et al., 2019).

#### Stability test

The stability test was accelerated in this study by the method cycling test. Syrups were tested on day 0 and after six cycles. One cycle consists of storage at 4±2 °C and 40±2 °C for 24 hours, respectively. Physicochemical evaluation, including organoleptic tests, pH, F value, and viscosity of syrup preparations (Reiger, 2000).

Organoleptic observations of syrup preparations included changes in color, aroma, consistency, and the appearance of gas or crystals. pH meter calibrated with standard pH solutions 4 and 10 before it

uses to measure the pH of syrups. The pH meter was placed in a bottle containing nanosilver syrup until the pH value was constant. Then the results are recorded and replicated three times. Viscosity measurement was done by dipping the spindle into the syrup preparation, the viscometer was turned on until the spindle was completely submerged and the measurement was constant. The syrup was put into the centrifugation tube, and then the centrifuge was operated at 3000 rpm for 10 minutes. Observation of the occurrence of phase separation was carried out. The F value was calculated by dividing the separation height with the total phase height (Ermawati, 2017).

### Formula of nanosilver syrup

**Table 1. Formula of nanosilver syrup with variation of syrup simplex concentration**

| Ingredients                 | Function              | Weight (gram)                 |                               |                               |
|-----------------------------|-----------------------|-------------------------------|-------------------------------|-------------------------------|
|                             |                       | Formula 1 (20% of syrup base) | Formula 2 (40% of syrup base) | Formula 3 (60% of syrup base) |
| Gembili's inulin nanosilver | Immunomodulator agent | 0.36                          | 0.36                          | 0.36                          |
| Simplex syrup               | Syrup base            | 12                            | 24                            | 36                            |
| Propylene glycol            | Co-solvent            | 4.8                           | 4.8                           | 4.8                           |
| Na benzoate                 | preservative          | 0.06                          | 0.06                          | 0.06                          |
| Distilled water add         | solvent               | 60 mL                         | 60 mL                         | 60 mL                         |

### Reduction glucose test

A standard glucose solution with a concentration of 0.04 mg/mL was taken and 1.0 mL of this solution was added to 1.0 mL of Nelson's reagent. The resulting mixture was then heated at 100 °C for 20 mins. After cooling, 1.0 mL of arsenomolybdate reagent and 7.0 mL of distilled water were added and vortexed until homogeneous. The absorbance of the resulting mixture was then measured at a wavelength ranging from 640-840 nm (Al-kayyis & Susanti, 2016). To create a standard curve, a total of 1.0 mL of glucose standard solutions with concentrations of 0.01, 0.02, 0.025, 0.03, 0.035, 0.04, and 0.05 mg/mL were added to volumetric flasks. Furthermore, 1.0 mL of Nelson's reagent was added, followed by heating at 100 °C for 20 mins and cooling. After cooling, 1.0 mL of arsenomolybdate reagent was vortexed until all precipitate dissolved. Subsequently, 7.0 mL of distilled water was added and shaken until homogeneous, and the absorption of each solution was measured at the maximum wavelength. A standard curve was constructed to show the relationship between the standard glucose concentration and the absorbance. For the analysis of nanosilver syrup chosen formula, a 1.0 mL solution with a concentration of 1.0 mg/mL was prepared and placed in a volumetric flask. In addition, 1.0 mL of Nelson's reagent was added, and the mixture was heated at 100 °C for 20 mins and cooled according to the procedure for determining the maximum wavelength.

### FT-IR analysis

FTIR was carried out to find information on the chemical bonds in the sample base syrup, nanosilver, and selected nanosilver syrup preparations, which can be seen from the wave number indicating the presence of functional groups. A typical infrared scan is produced in the mid-infrared region of the light spectrum. The mid-infrared region is a 400-4000  $\text{cm}^{-1}$  wave number, with the same wavelength of 2.5-25 microns (10-3 mm) (Sanjiwania & Sudiarsa, 2021). FT-IR analysis ensured that the inulin nanosilver was still in the syrup preparation. (Melanie et al., 2015) state that wave numbers below 900  $\text{cm}^{-1}$  are characteristic of inulin carbohydrates.

### Data Analysis

Data from the physicochemical properties test for nanosilver syrup between formulas were analyzed using the SPSS 21 program through the Shapiro-Wilk test. If the data were normally distributed, proceed with the One-Way ANOVA test to determine a significant difference between the three formulas. The One-Way ANOVA test data results showed that significant differences would be continued with the Post Hoc test. The results of the physicochemical properties for each formula after the stability test were analyzed using the SPSS 21 program through the Paired Samples T-Test to determine a significant difference.

### RESULT AND DISCUSSION

Determination is carried out to prove the correctness of the sample used for research. Determination Results with letter No. 130/UN27.9.6.4/Lab/2022 stated that the tubers used in this study were true yam tubers of the species *Dioscorea esculenta* (Lour.) Burkill.

Biosynthesis or green synthesis is an environmentally friendly and safe silver nitrate synthesis method using biological materials, microorganisms, and plants. This method is safer, more cost-effective, and environmentally friendly than physical and chemical methods. Biosynthesis using plant extracts is simpler than microorganisms because there is no need to prepare microorganism media or cell culture (Pulit-Prociak et al., 2015). Silver metal's physical method is breaking metal solids into small nano-sized particles, while the chemical method is carried out by forming nanoparticles through chemical reactions (Lembang, 2013). Using chemical-reducing agents has disadvantages, such as producing hazardous waste, expensive reducing agents, and being toxic. The nanosilver formed is also unstable because no capping agent can be adsorbed or bind to the surface of the nanoparticles to prevent agglomeration (Abou El-Nour et al., 2010). Development of bioreduction methods using plant extracts as an alternative to those two methods (Lengke et al., 2007).

#### Characteristic of Gembili's inulin nanosilver

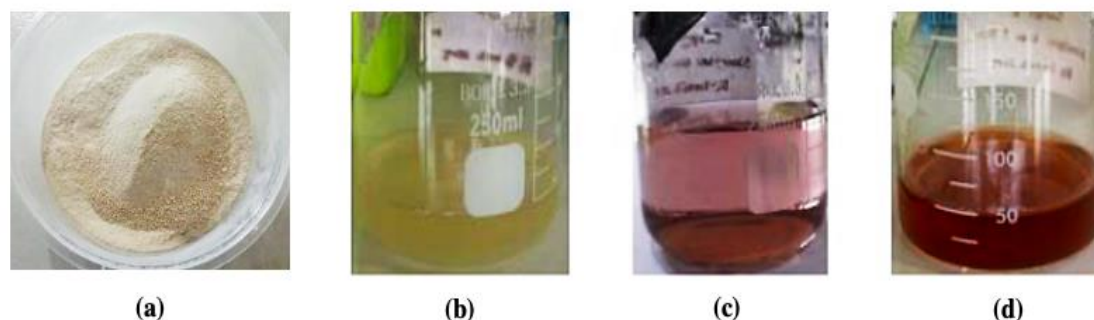
The biosynthesis process was carried out at 60 °C for 15 minutes. The optimal temperature determines the size of the nanosilver formed (Ermawati et al., 2021). Biosynthesis at 60 °C for gembili's inulin nanosilver, the reaction will be completed more quickly, and the resulting particle size is smaller than the biosynthetic process carried out at room temperature. The presence of groups –OH contained in inulin acts as a capping agent, reducing Ag<sup>+</sup> to Ag<sup>0</sup>, thus producing a stable nanosilver. Nanosilver synthesis produces a brownish solution color (Figure 1); this change indicates a reduction process (Ag<sup>+</sup>) when bound to the -OH functional group contained in the compound in inulin in Gembili tuber. The ability of compounds in plants to reduce (Ag<sup>+</sup>) into Ag<sup>0</sup> nanoparticles is the working principle of plants in reducing nanosilver. The maximum wavelength obtained from UV-Vis spectrophotometric characterization is 440 nm. These results indicate that nanosilver has been formed because nanosilver produces absorbance peaks in the SPR (Surface Plasmon Resonance) range of 400-500 nm (Kumar & Yadav, 2009).

Particle Size Analyzer (PSA) to determine particle size distribution and uniformity. The data obtained from this measurement are Z-average, PDI (Polydisperse Index), and potential zeta. Z-average is the average particle diameter, and PDI is the magnitude of the particle size distribution distance. PDI shows that the distribution distance of the sample is wide and has more than one peak; the particle size is varied or heterogeneous (Murano, 1998). Zeta potential is a parameter of the electric charge between colloidal particles (Ozturkoglu-Budak et al., 2019).

The Z-average of the gembili's inulin nanosilver is 109.3-128.9 nm. According to (Nagpal et al., 2010), nanoparticles are solid colloidal particles with a 1-1000 nm diameter. The results indicate that the size of the gembili's inulin nanosilver met the range of nanoparticles. The PI value <0.5 indicates that the resulting nanosilver has a homogeneous particle size distribution. Furthermore, the zeta potential values obtained are -29.1 to -28.4 mV. Zeta potential value indicates that the strength of the particles to repel each other is getting stronger to produce a stable dispersion of the preparation. The



zeta potential value of a stable preparation is more than +25 mV or less than -25 mV. The zeta potential value in this study is less than -25 mV indicating that the resulting nanosilver is stable.



**Figure 1. The results of gembili's inulin powder (a); gembili's inulin solution at 40 °C (b); silver nitrate solution (c); and gembili's inulin nanosilver at 60 °C (d)**

#### **Inulin's nanosilver effective dose**

This study converted a 4 mg/kgBW dose into a 70 kg human dose. The 4mg/kgBW dose was based on Nuraini's research (2022), which found an effective dose of inulin's nanosilver as an immunostimulator. (Nathaya, 2023) carried out an acute toxicity test at this inulin's nanosilver dose to obtain the LD50 value using the Thompson-Weil method. During observation, 24 hours after administration of the sample, it did not show the achievement of 50% mortality in the test animals. The number of deaths of the test animals shows that the f factor was not obtained from the Thompson-Weil table, so the LD50 value could not be calculated. This means that it is safe to take orally at a dose of 4 mg/kgBW.

Nanosilver syrup is formulated for health supplements. Health supplements are products intended to supplement nutritional needs, maintain health, and have nutritional value and physiological effects. Supplement products also contain vitamins, minerals, amino acids, and other non-plant ingredients that can be combined with plants. The rules for using this nanosilver syrup preparation are 5 mL once a day. The minimum use of nanosilver is 10 mg/kgBW or equivalent to 78 mg after being converted to a 70 kg human. A study by (Grzegorz et al., 2013) proved that giving honeybees nanosilver syrup containing 25 mg/L nanosilver could improve bees' health. Based on the results of the preparation stability test, it was found that the best formula was F3 with a 60% simplex syrup concentration.

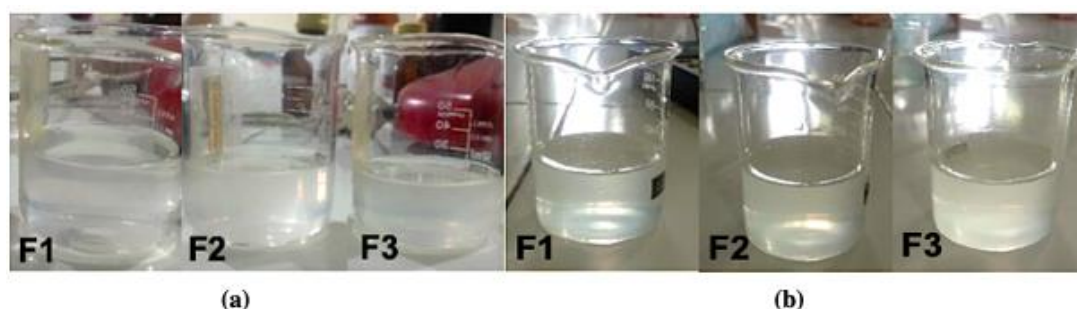
#### **Stability test of Gembili's inulin nanosilver syrup**

Syrup simplex is a sweetener as an additive in syrup, and a sweetener is added to give a sweet taste to the syrup. Stabilizers are meant to keep the syrup stable; examples of stabilizers are antioxidants, buffers, and complexes (Fickri, 2019). The higher the concentration of simplex syrup used, the higher the consistency of the resulting preparation. The addition of the concentration of the simplex syrup used did not affect the color of the nanosilver syrup but could affect the clarity of the color (Figure 2). The odor parameter produced from the nanosilver syrup preparation comes from the sweetener of simplex syrup, which is typical and pungent, so the increase in the concentration of simplex syrup does not affect the odor. The taste produced from the nanosilver syrup preparation comes from the sweet simplex syrup sweetener, so the increase in the concentration of simplex syrup in the nanosilver syrup formula does not affect the taste of the preparation.

**Table 2. The result of physicochemical stability test of gembili's inulin nanosilver syrup during the various temperature (4°C and 40°C) for six cycle**

| Physicochemical test         | Cycling test at 4±2°C and 40±2°C for six cycle |                        |                                  |                          |                                  |                          |
|------------------------------|--|------------------------|----------------------------------|--------------------------|----------------------------------|--------------------------|
|                              | Formula 1 (20% of simplex syrup)               |                        | Formula 2 (40% of simplex syrup) |                          | Formula 3 (60% of simplex syrup) |                          |
|                              | before   | after                  | before                           | after                    | before                           | after                    |
| <b>pH</b>                    | 4.87±0.02                                      | 4.74±0.04 <sup>a</sup> | 5.17±0.02                        | 5.08±0.04 <sup>b</sup>   | 5.25±0.03                        | 5.17±0.05                |
| <b>Viscosity (cps)</b>       | 66±2.1   | 60±1.5 <sup>c</sup>    | 78±2.5                           | 71±2.1                   | 92±2.6                           | 84±2.6 <sup>d</sup>      |
| <b>Reduction glucose (%)</b> | 13.88±0.002                                    | 12.21±0.002            | 11.74±0.001                      | 16.41±0.002 <sup>e</sup> | 13.68±0.002                      | 20.59±0.002 <sup>f</sup> |

\*mean±SD, replication three times; <sup>a,b,c,d,e,f</sup> significant different



**Figure 2. The result of organoleptic evaluation of gembili's inulin nanosilver syrup before (a) and after (b) cycling test. Where F1 (20% of simplex syrup), F2 (40% of simplex syrup), and F3 (60% simplex syrup)**

The recommended pH value for syrup preparations is 4–8 (Kementrian Kesehatan RI, 2014). It is necessary to observe the pH because if the syrup formed is too acidic, it can irritate the stomach, while if it is too alkaline, it will cause a bitter taste. The pH of simplex syrup is around 7, so formula 3 has a higher pH than formula 1 and formula 2 (Table 2). Changes in pH in three formulas still meet the requirements. The pH value has decreased but no more than 50% during storage (Deviarny et al., 2013). After stability testing on storage, the three formulas with temperature variations of 4°C and 25°C showed the same pH value based on the recommended syrup pH range according to the Indonesian Pharmacopeia (Kementrian Kesehatan RI, 2014), which requires a pH of 4 – 8. Based on the test results, it can be concluded that an increase in the concentration of simplex syrup does not affect the pH of the nanosilver syrup preparation. Analysis of the homogeneity of variance test obtained a significance value of >0.05 (p-value = 0.763), meaning there is a variance similarity between groups. The One-way ANOVA test obtained a significance value of <0.05 (p-value = 0.000). There is a significant difference between groups in the pH stability test of the preparation.

The viscosity of preparation is also influenced by mixing or stirring factors during the formulation process. The stirring of syrup causes the droplet particles colloide to move freely so that the homogeneity of active substance increases. The joining of droplet particles will result in the contact area between droplet particles becoming weaker. The consistency that decreases in the system will result in a decrease in viscosity in the system during storage (Ozturkoglu-Budak et al., 2019). The difference in viscosity values can be affected by the amount of added solid particles (sugar) in the media. The higher the number of sugar particles, the greater the viscosity value of the syrup (Fajri et al., 2017). According to (Ozturkoglu-Budak et al., 2019), viscosity has a relationship that is directly proportional to the total amount of dissolved sugar. Syrup viscosity can occur because of the strong bonds between particles; the stronger the bonds between particles, the higher the viscosity value. The presence of hydrogen bonds between the hydroxyl groups (OH) on the sugar molecules and the water

*The effect of ... (Ermawati and Hanuriansyah)*

molecules causes the high viscosity of the syrup (Grzegorz et al., 2013). These results follow the standard syrup preparation, which is determined to have a viscosity of 27 cPs - 396 cPs (Kementrian Kesehatan RI, 2014). Nanosilver syrup with a greater concentration of simplex syrup has a higher viscosity. The homogeneity of variance test analysis obtained  $\text{sig} > 0.05$  ( $p\text{-value} = 0.876$ ). Which means there is a similar variance between groups. One way ANOVA test obtained a significance value of  $<0.05$  ( $p\text{-value} = 0.000$ ). There is a significant difference between the viscosity and the formula. So, this shows that variations in the concentration of simplex syrup affect the viscosity value of nanosilver syrup preparations.

### Reduction glucose of nanosilver syrup

Reducing sugar test for nanosilver syrup preparations with the Nelson-Somogyi method, using Spectrophotometer UV-VIS. The principle of the Nelson-Somogyi method is one method of testing glucose concentration with the mechanism of the Nelson reagent will oxidize glucose and then form a molybdenum complex that is blue green after adding arsenomolybdate reagent (Razak et al., 2012). The intensity of the color formed indicates the amount of reducing sugar present in the sample, and this is because the concentration of reduced arsenomolybdate is proportional to the concentration of copper (I) oxide ( $\text{Cu}_2\text{O}$ ), while the concentration of  $\text{Cu}_2\text{O}$  is proportional to the concentration of reducing sugar (Al-kayyis & Susanti, 2016). The maximum wavelength in this research was carried out in the wavelength range of 400-800 nm. In measuring the maximum wavelength of the standard glucose solution, a maximum wavelength of 758 nm was obtained, which was then carried out to determine the standard glucose curve and to analyze the concentration of the nanosilver syrup. Research conducted by (Sari, 2019) obtained a maximum wavelength of 761 nm. This difference still meets the requirement of the tolerance limit, which is approximately 1-3 nm (Kementrian Kesehatan RI, 2014).

The standard curve results obtained by the equation  $y = 0.0309x - 0.097$  are used to calculate the reducing sugar content in the nanosilver syrup sample where (y) is the absorbance value, and (x) is the reducing sugar content in the sample. The correlation coefficient ( $r^2$ ) obtained is 0.9465, which indicates a linear relationship between concentration and absorbance. A good value of  $r^2$  close to 1 indicates a linear correlation between concentration and absorbance. The percentage of reducing sugar of formula 2 and formula 3 increased after stability test, the increase in reducing sugar levels is due to the inverse process of sucrose, and the process into reducing sugar increases when the concentration of sucrose increases, besides that during heating, the process of hydrolysis of sucrose into reducing sugars (glucose and fructose) occurs (Table 2) (Shafie et al., 2022).

### FT-IR Analysis

Fourier Transform Infrared (FTIR) is a tool used to detect the functional groups of a compound. The working principle of FTIR is to collect infrared light, which is spread on the surface of the sample to determine the frequency of the absorbed waves, which will then be interpreted to determine the specific group of the sample. This study evaluated the chemical interaction between inulin and  $\text{AgNO}_3$  using FTIR spectroscopy at wavenumber  $500\text{--}4000\text{cm}^{-1}$ . FTIR analysis was carried out on samples of biosynthetic solutions inulin- $\text{AgNO}_3$  (nanosilver), selected nanosilver syrup preparations, and syrup preparations without nanosilver. The interpretation of the FTIR spectrum results is then assisted by a correlation table (Table 3) showing the type of functional group from the range of wavenumbers.

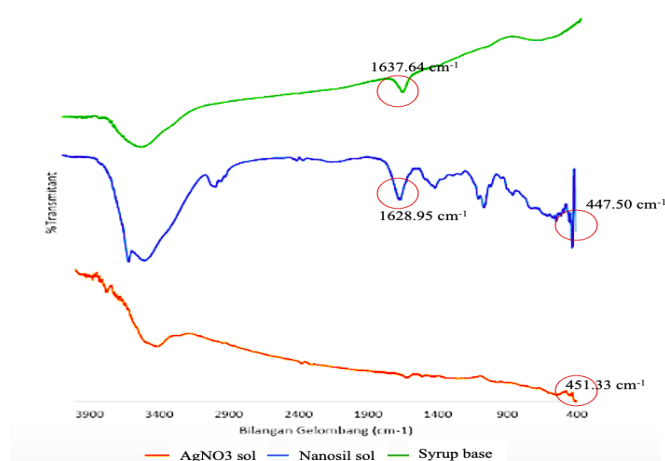
There is a similarity in the absorption peaks between the nanosilver inulin solution and the nanosilver syrup. In nanosilver inulin solution, there is an absorption peak of  $1616.42\text{ cm}^{-1}$  ( $\text{C}=\text{C}$ ) which is also present in nanosilver syrup of  $1628.95\text{ cm}^{-1}$  ( $\text{C}=\text{C}$ ) (Figure 3). (Melanie et al., 2015) stated that inulin derived from dahlia tubers has identical FTIR spectra with chicory inulin and artichoke inulin, which is characterized by the appearance of an O-H stretching absorption peak at wave number  $3300\text{ cm}^{-1}$ , which is a characteristic of inulin. In the nanosilver syrup sample, there was an absorption peak of  $425.32$ ;  $447.50\text{ cm}^{-1}$  ( $\text{Ag-N}$ );  $535.27\text{ cm}^{-1}$  (pyranose ring), which was also



present in the inulin's nanosilver of 412.78; 451.326  $\text{cm}^{-1}$  (Ag-N); 552.63  $\text{cm}^{-1}$  (pyranose ring). Ag-N groups (silver ions) exist in nanosilver solution and nanosilver syrup because silver ions ( $\text{Ag}^+$ ) are the basic material used to produce nanosilver particles. These results prove that nanosilver syrup still contains nanosilver characterized by the presence of Ag-N groups. In nanosilver biosynthesis, the reducing agents, such as organic compounds or compounds from plant extracts, can interact with silver ions ( $\text{Ag}^+$ ) and form AgN groups. These compounds act in reducing silver ions into nanosilver particles. There are wave shifts in the nanosilver solution, and nanosilver syrup samples indicate that there has been an interaction between the functional groups and the silver nanoparticles, which may affect adding ingredients. This interaction will produce a combination of properties (new properties) that are different from the properties of the original material.

**Table 3. The result of functional group of silver nitrate solution, syrup base and nanosilver syrup by FT-IR analysis**

| Functional group | Wave Number ( $\text{cm}^{-1}$ ) |            |                                   |
|------------------|----------------------------------|------------|-----------------------------------|
|                  | Silver nitrate Solution          | Syrup base | Gembili's Inulin Nanosilver Syrup |
| Ag-N             | 451.326; 412.78                  |            | 447.50; 425.32                    |
| Pyranose ring    | 552.63                           |            | 535.27                            |
| C-C vibration    | 702.12                           | 717.55     | 716.59                            |
| OH- bending      | -                                | 1384.95    | 1385.91                           |
| C-O-C            | 1169.88                          | -          | 922.98; 841.00                    |
| OH-bending       | -                                | -          | 1082.11; 1042.57;<br>994.35       |
| $\text{NO}_2$    | -                                | -          | 1268.25; 1205.56                  |
| C=C              | 1616.42                          | 1637.64    | 1628.95                           |
| O=C=O stratching | 2374.47; 2306.00                 | 2356.15    | 2355.19; 2312.75;<br>2108.29      |
| C-H stratching   | 2939.64; 2868.27                 | -          | 2924.21; 2886.60;<br>2854.77      |
| O-H stratching   | 3396.79; 3249.23                 | 3451.76    | 3534.71; 3421.8                   |



**Figure 3. The FT-IR spectra of silver nitrate solution, syrup base and nanosilver syrup where x= wave number ( $\text{cm}^{-1}$ ) and y= % transmittant**

## CONCLUSION

The results showed that the concentration of simplex syrup affected consistency, pH, and viscosity. The simplex syrup concentration of 60% met the requirement with a medium thick consistency, pH  $5.25 \pm 0.03$ , a viscosity of  $92 \pm 2.6$  cps, reducing sugar content was  $20.59\% \pm 0.002$ , and the FTIR profile showed that it still contained nanosilver which was indicated by the presence of Ag-N groups compared to silver nitrate solution.

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