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# A Novel of Green Synthesis Silver Nanoparticles Using Kecemcem Leaves (*Spondias pinnata* (L.F) Kurz.) Extract for Tetracycline Biosensor

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**Abstract:** Tetracycline is one of antibiotics that widely used inappropriately in livestock. The long-term use of tetracycline may cause bacterial resistance. Biosensor can be applied as one of the detection methods for antibiotics in livestock. Development of biosensor materials can be obtained by nanoparticles formation through a synthesis process. Kecemcem leaves are materials that can be used to synthesize silver nanoparticles. This study aimed to synthesize silver nanoparticles using kecemcem leaves (*Spondias pinnata* (L.f) Kurz.) and its application as tetracycline biosensor. Aqueous extract of kecemcem leaves and 1 mM $AgNO_3$ solution were combined in a 1:10 (v/v) ratio to create silver nanoparticles. The biosensor capability of silver nanoparticles was evaluated against tetracyclines after they were characterized using a UV-Vis spectrophotometer, particle size analyzer, scanning electron microscope-EDS, transmision electron microscope, and FTIR spectrophotometer. The maximum wavelength of the AgNPs scanning results on the UV-Vis spectrophotometer was in the 405 nm region. The results of characterization on TEM, SEM-EDS and PSA morphology of the formed AgNPs were spherical, the smallest particle size was 26.886 nm on average. In the characterization using FTIR there were found hydroxyl groups in AgNPs. The resulting nanoparticles could act as tetracycline biosensors, as evidenced by the shift in wavelength when AgNPs interacted with tetracycline. Detection of tetracycline was established based on the SPR technique. The brownish red color of the AgNPs solution turned to clear slightly reddish in the presence of tetracycline, accompanying the decrease in intensity of SPR band. Based on the method validation carried out, the linearity value was obtained in the form of r value of 0.983, LOD value of 0.879 ppm, and LOQ of 2.93 ppm, while a precision value of 3.356%, and an accuracy of 82.58%.

***Keywords:*** *Biosensors, green synthesis, kecemcem leaves, silver nanoparticles, tetracyclines.*

## 1. Introduction

Tetracyclines are antibiotics used for respiratory and digestive tract infections in human. Tetracyclines are also used for the treatment of livestock, thereby reducing the risk of death and returning livestock to a healthy condition. Tetracycline is irrationally frequently used as a feed supplement since it maximizes broiler development and boosts feed intake efficiency (Widiasih *et al*., 2019). Extensive use without following indications can lead to accumulation of tetracycline residues in livestock organs. Over 75% of the tetracycline utilized will be released into the environment in an active form through feces and urine. Tetracycline which is used globally has led to findings of tetracycline contamination in water sources, and the toxicity of tetracycline contamination in water sources has a more dangerous level than parasitic fish and *Daphnia sp*. (Xu *et al*., 2021) can cause serious harm to human health who consume livestock and water contaminated with tetracyclines.

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In Indonesia, the residue of tetracycline antibiotics was found in pig feces as much as almost 95%, pig farm waste as much as 66.7% and pig slaughterhouse wastewater as much as 50% (Pazra *et al.,* 2023). In free-range and laying hens there were findings of meat containing antibiotic residues, especially tetracyclines of 12.5% (Widiasih *et al.,* 2019). These findings prove that tetracycline is still widely used and contained in parts of livestock in several provinces in Indonesia. In Indonesia, to prevent this case from occurring, the maximum residue limit for tetracycline in livestock products must comply with the safe limit in meat and eggs of 0.05 mg/kg, so many efforts have been made to develop a system for detecting tetracycline residues in livestock parts (Widiasih *et al*., 2019).

A method that can detect an antibiotic in livestock parts is a biosensor (Ghodake *et al*., 2020). Tetracyclines and other antibiotics can be detected using biosensors (Jalalian *et al.,* 2018). According to (Kim *et al.,* 2010) who stated that tetracycline can be detected by biosensors derived from gold nanoparticles, but this method is difficult to develop given the price of gold which is quite expensive and the stability of the nanoparticles is not good. Antibiotics can be easily, quickly, and affordably detected by biosensors in tainted food products as well as pharmaceutical preparation. Silver nanoparticles are one of the materials that can be developed as a biosensor (nanoprobe). Antibiotics analysis with AgNPs can be carried out by visual observation (colorimetry)

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and observation of the shift in the AgNPs wavelength on a UV-Vis spectrophotometer (Ghodake *et al.*, 2020). Nano-based biosensors have better biosensor characteristics (Malhotra & Ali, 2018). Silver nanoparticles have unique properties, it exhibits as an efficient sensor probe with enhanced sensitivity and selectivity (Gurunathan *et al.,* 2016; Haes *et al*., 2002). During the sensing process, the silver nanoparticles exhibit a visible color change in the presence of particular external agents including ions or antibiotics. The visual color change suggests that the targeted ions or molecules are present in the analysis. Therefore, herein, efforts are being made in developing silver nanoparticles based sensors which are cost-effective, easy and rapid monitoring system to detect hazardous antibiotics in environmental samples (Singh *et al*., 2018)

In applying the benefits of silver nanoparticles as a biosensor, synthesis is necessary. The chemical synthesis method using biological compounds is a method that can be applied in the nanoparticles preparation (Deng *et al*., 2016). This method is broadly utilized, because it has the advantages of being cheap, easy to carry out, sustainable, and renewable. Plant extracts are the most commonly used ingredients. Apart from being cheap, abundant and environmentally friendly resources are also advantage of plant extracts (Sharma *et al*., 2019).

One of the plants that can be utilized in the production of silver nanoparticles is kecemcem leaves (*Spondias pinnata (L.f)* Kurz.). It has several other names, including cemcem and forest kedondong. The distribution of kecemcem plants in Bali is dominantly found in Kintamani, Bangli and some can be found in other areas of Bali such as Gianyar and Klungkung. The local community grows kecemcem plants along the fields or on the edges of rice fields which can play a role in reforestation and as a traditional health drink (loloh). However, the use of kecemcem leaves is not optimal, so the selling value of these leaves is very low (Sujarwo *et al*., 2017).

It has been demonstrated that silver nanoparticles can be biosynthesised utilizing kecemcem leaf bioreductors. Based on research by (Purnamasari *et al*., 2021) optimal AgNPs results are formed at 60°C with extract concentration of 0.5%. This description suggests that the bioreductor found in kecemcem leaves was used as reducing/stabilizing agent for the production of stable, small size AgNPs. Nanoparticles were characterized by various analytical methods. The possible

application of AgNPs as rapid, selective, easy-to-use and cost effective colorimetric sensors for tetracycline was investigated.

## 2. Materials and Methods

### Kecemcem Leaf Sample Preparation

After being cleaned under running water, the kecemcem leaves were chopped into small pieces and allowed to dry for seven days away from the sun. Kecemcem leaves were dried, ground into a powder, and then sieved through a 50 mesh screen. In a 250 mL beaker, 20 grams of powdered kecemcem leaf and 100 mL of demineralized aqua were boiled for 15 minutes at 60°C. The mixture was then allowed to cool before being filtered through filter paper. The filtrate is concentrated to 0.5 percent and utilized to create silver nanoparticles.

### Phytochemical Test

The purpose of the phytochemical screening test is to identify secondary metabolites present in kecemcem leaves. Alkaloids, tannins, terpenoids/steroids, flavonoids as well as saponins were all examined in the phytochemical screening test of the kecemcem leaf aqueous extract.

### Silver Nanoparticle Synthesis

Weighing 170 mg of $AgNO_3$ powder, dissolving it in aquadem in a beaker glass, and then placing it in a 1000 mL volumetric flask, demineralized aqua was added, and the mixture was shaken until it was homogenous. This produced a 1 mM $AgNO_3$ solution. A 0.5% kecemcem leaf water extract was combined with a 1 mM $AgNO_3$ solution. Kecemcem leaf water extract and $AgNO_3$ solution had a 1:10 (v/v) ratio. The mixture was heated to 60°C until it turned a brownish red color. Subsequently, the AgNPs that has been obtained was measured for its wavelength and absorbance on UV-Vis spectrophotometry and can then be characterized using PSA, SEM-EDS, FTIR, and TEM instruments.

### Silver Nanoparticles Characterization

The purpose of the UV-Vis spectrophotometer's characterisation test is to ascertain whether silver nanoparticles have developed. The AgNPs sample was put into the cuvette and the absorbance was examined. The maximum wavelength between the range of 400 – 450 nm indicates that the sample contains AgNPs. The functional groups of the secondary metabolites in the pure kecemcem leaf water extract and the colloidal silver nanoparticles, which were produced from the aqueous extract of kecemcem, were compared using FTIR analysis.

The morphology as well as size of the resultant pictures were ascertained using TEM characterization. A few drops of colloidal silver nanoparticles were dropped on the TEM grid and then analyzed. Particle sizes range from 1-100 nm was determined wheter it has spherical, triangular, crystalline or anisotropic particle shapes. The goal of SEM-EDS characterization is to observe the composition and shape of colloidal silver nanoparticles. A precipitate was created by centrifuging the sample solution for ten minutes at 10,000 rpm, washing the resultant precipitate with ten milliliters of aqua demineralized, and then centrifuging it once more. After that, the precipitate was dried at 50°C in an oven. SEM-EDS was then used to evaluate the resulting powder. The PSA characterisation test seeks to determine the zeta potential of silver nanoparticles as well as their size or diameter. A PSA device was used to examine the AgNPs solution after it had been placed in a cuvette. The resulting size ranges from 0.6 nm – 7 µm.


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**AgNPs Analysis as a Tetracycline Biosensor**

A concentration series solution of tetracycline 1; 2; 3; 4; 5; 6; 7; 8; 9; and 10 ppm were prepared with 0.1 N HCl as solvent. The 1.8 mL of demineralized water and 0.2 mL of AgNPs were combined with each tetracycline concentration. Then the mixture was analyzed for its wavelength and absorbance on a UV-Vis spectrophotometer. The shift between the peak wavelengths of AgNPs and the wavelengths of the mixture of nanoparticles and tetracycline (MNT) is an indication of a sensory response by AgNPs to tetracyclines.

**Method Validation**

Method validation was carried out to ensure and confirm the method used is good and can be used continuously. Method validation includes: linearity, determination of LOD and LOQ, determination of precision, and determination of accuracy in MNT (Metal Nanoparticles Tetracycline).

## 3. Results and Discussion

**Silver Nanoparticles Synthesis**

The extraction process made an initial concentration of 20%, then diluted to a concentration of 0.5% which was then used for synthesis. Heating was carried out on the extract for 15 minutes at 60°C, which aims to speed up the extraction process. This method was selected since it is simple, affordable, and doesn't call for specialized equipment. A chemical found in plant extracts may become more soluble if the water temperature is raised during the extraction process (Sulaiman *et al*., 2017). While the steroid test yielded negative results, the qualitative test using phytochemical screening on an aqueous extract of kecemcem leaves revealed that the sample included terpenoids, flavonoids, tannins, and alkaloids and saponins (Figure 1). This positive result occurred due to the suitability of the solvent during the extraction process. The appropriate solvent is one of the factors that influence whether the desired compound is dissolved well or not (Sulaiman *et al*., 2017). The solvent used is aqua demineralization which is polar, so the extract obtained is polar. The results of flavonoids and tannins are more concentrated because these compounds are polar, so they dissolve in polar solvents. Alkaloids, terpenoids, and saponins also showed positive results, but the results were not very concentrated or significant. This is probably because there are not many of these substances in the kecemcem leaf aqueous extract. Because the steroid component is non-polar, it is insoluble in solvents and does not show up in the steroid test on the aqueous extract of kecemcem leaves, which is why the test yielded negative results. The amount of steroid content that is not much makes this steroid compound must be tested more specifically to see the quantity (Swathi & Lakshman, 2022).

Based on studies on the synthesis process, it was evident that the $AgNO_3$ solution containing kecemcem leaf water extract changed color; initially yellow, the solution turned brownish red when heated, as seen in Figure 2. One sign that silver nanoparticles are forming is this change in appearance. This is one of the features of the reduction process in silver ions that results in a color shift and the production of silver nanoparticles (Badi'ah *et al.,* 2019).

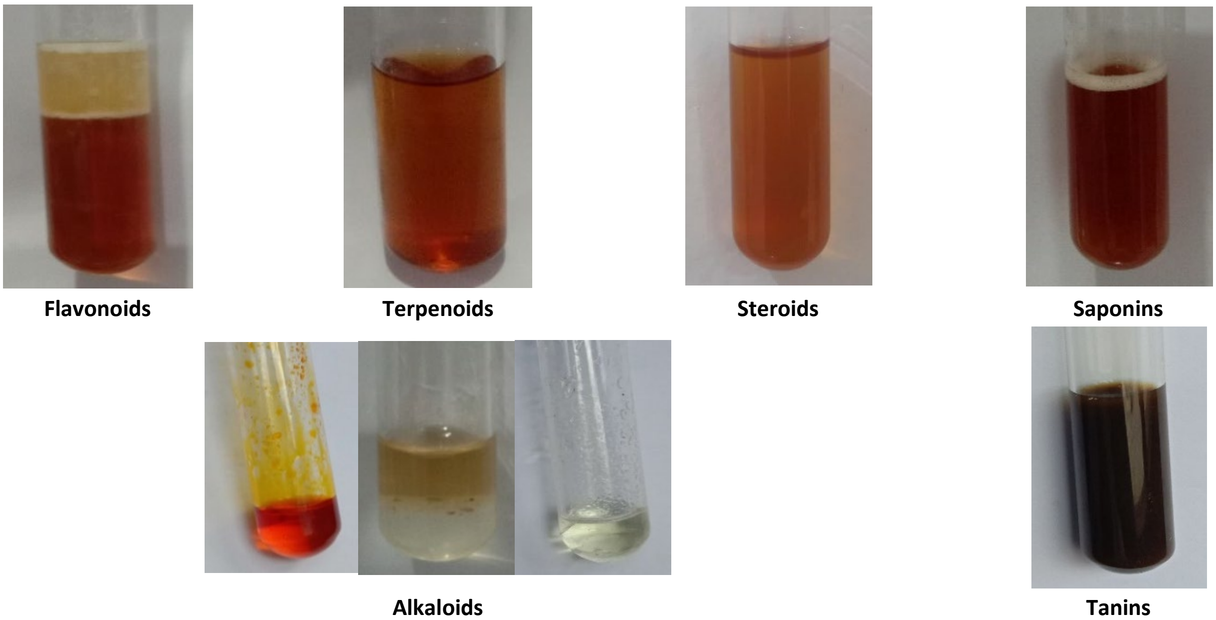


**Figure 1.** Phytochemical screening on aqueous extract of kecemcem leaves.


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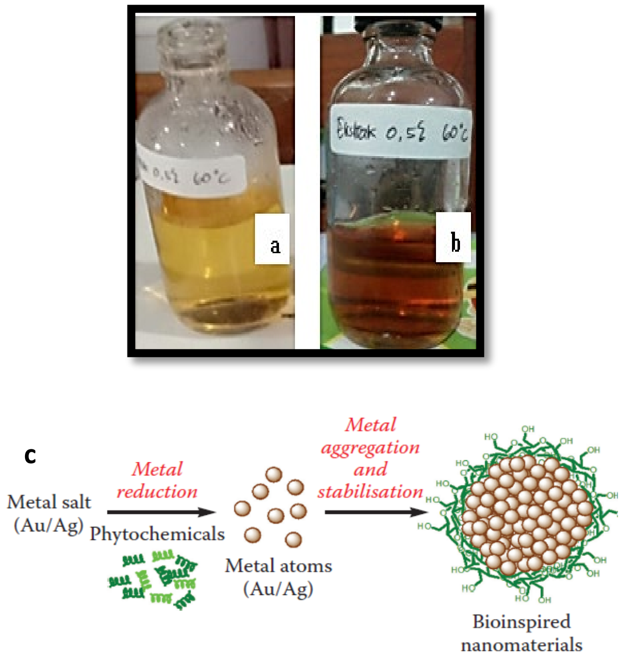


**Figure 2**. Formation of AgNPs colloid (a) initial mixing between $AgNO_3$ solution with plant extract, (b) formation of AgNPs after synthesis for 15 minutes, and (c) AgNPs formation scheme (Bhaumik *et al.,* 2015).

**Silver Nanoparticles' Characterization**

The study's findings demonstrated that silver nanoparticles created by reducing kecemcem leaf aqueous extract exhibited SPR intensity at a wavelength of 400-450 nm, with a maximum wavelength of 405 nm, as measured by a UV-Vis spectrophotometer (Figure 3). The fact that the produced silver nanoparticles' SPR intensity satisfied the necessary conditions for nanoparticle production indicates that the synthesis was successful.

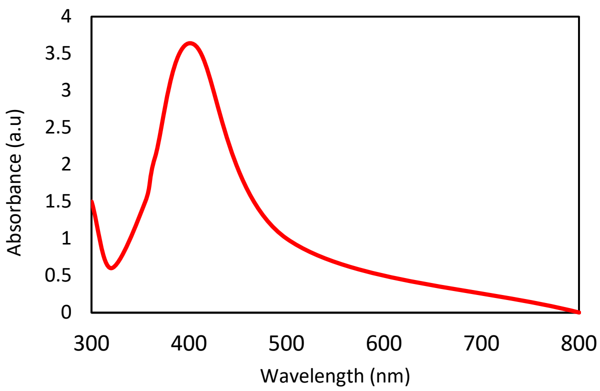


**Figure 3**. AgNPs absorbance curve

Characterization of aqueous extract of kecemcem leaves with FTIR showed absorptions at certain wavenumbers which are characteristic of flavonoid compounds that are similar to the flavonol group (Figure 4a). Absorption occurs in several areas, namely at 3626.50 $cm^{-1}$ and 3460.06 $cm^{-1}$ indicating -OH vibrations of the hydroxyl groups and C-H aromatics group. A chromophore group is present in the tested sample when the wave number is 2075.12 $cm^{-1}$ (Suchithra *et al*., 2021). Absorption at wavenumbers 562.14 $cm^{-1}$ and 501.04 $cm^{-1}$ shows the existence of phenolic compounds (Niraimathi *et al*., 2013), while the presence of absorption in the 1633.96 $cm^{-1}$ area suggests C=C stretching of the alkenes group and C=O stretching of the ketone group (Periasamy *et al*., 2022) .

Multiple absorptions in specific regions, which suggested the existence of flavonoid compound characteristics, were the findings of the characterization analysis on AgNPs (Figure 4b). The presence of absorption in the 3445.32 $cm^{-1}$ range is indicative of the -OH group, which acts as a reducing agent. In addition to -OH, aromatic C-H also absorbs in this region. An absorption in the 1634.01 $cm^{-1}$ region indicates C=C stretching of the alkenes group as well as C=O stretching of the ketone group (Periasamy *et al.,* 2022), a wave number of 2066.73 $cm^{-1}$ demonstrates the presence of a chromophore group (Suchithra *et al*., 2021), and at a wave number 562.64 $cm^{-1}$ showing the presence of silver ions' presence (Niraimathi *et al*., 2013).

According to the preceding FTIR study results on the characterization of the aqueous extract of kecemcem leaves, flavonoid compounds with -OH groups are present and can donate electrons to create AgNPs. According to earlier studies (Bhaumik *et al*., 2015; Ghodake *et al*., 2020; Mittal *et al*., 2014) the presence of hydroxyl groups (-OH), which were demonstrated in the characterization of AgNPs, could function as capping agents by generating hydrogen bonds.


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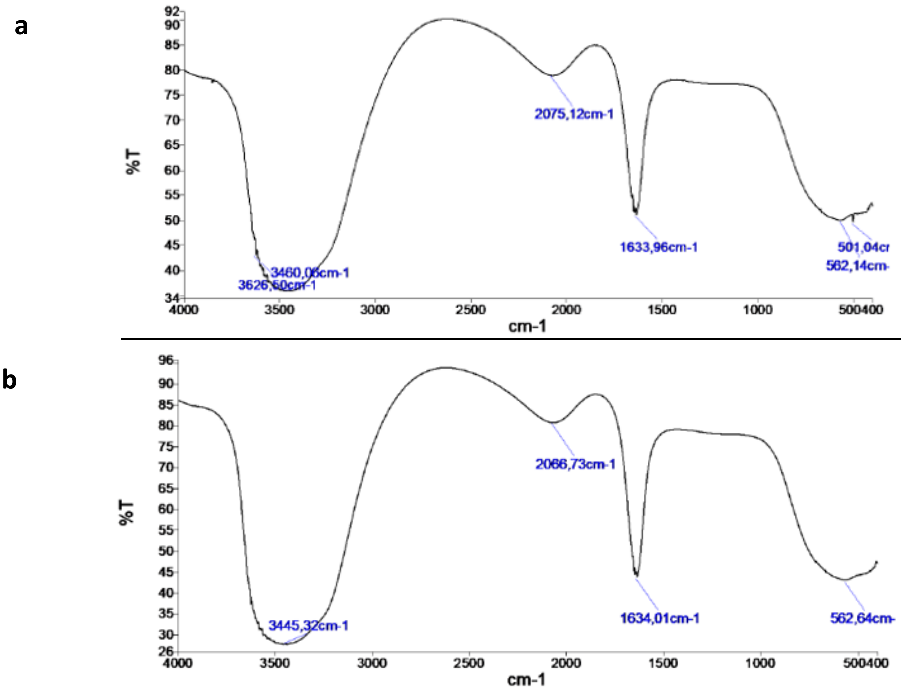


**Figure 4**. FTIR Spectrum Results from (a) aqueous extract of kecemcem leaves and (b) AgNPs.

A strong method for high-resolution imaging of a thin layer on a solid sample for structural as well as compositional investigation of nanomaterials is TEM (Mittal *et al*., 2014). AgNPs made from kecemcem leaf extract were shown in a TEM micrograph to be spherical, around 100 nm in size, and capped with plant components that inhibited particle aggregation (Figure 5). The size of nanoparticles made from plant bioreductors ranges from 1 to 100 nm, and they have a spherical morphology, smaller the size of AgNPs, the more active they are (Restrepo & Villa, 2021).

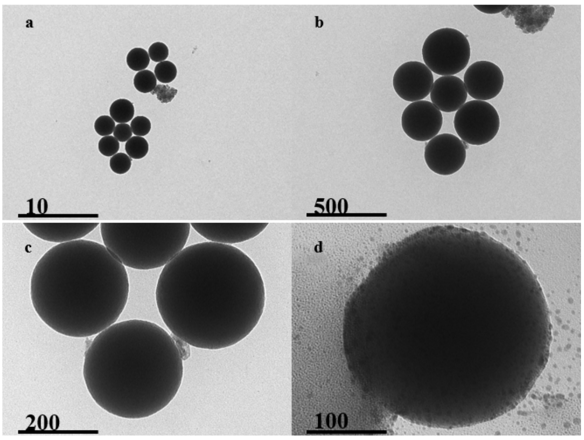


**Figure 5**. AgNPs morphological analysis using TEM at magnification of (a) 10,000x, (b) 20,000x, (c) 50,000x and (d) 100,000x

The results of SEM measurements (Figure 6.) provide an overview of the formation in the form of micro to $Ag^0$ nanoparticles in the synthesized colloid. The SEM image at 30x magnification shows random-shaped crystal particles with a fairly large size (Figure 6a). In contrast to the SEM at 10,000x magnification, Figure 6d shows randomly shaped particles with a size range of 1µm that are close to each other (agglomeration). Based on the research of (Restrepo & Villa, 2021) the results of the morphology of silver nanoparticles are random and diverse, this can be caused by the aggregation effect of nanoparticles which can cause agglomeration (Restrepo & Villa, 2021). The agglomeration that occurs causes large nanoparticles, and this is influenced by the presence of Van der Waals forces.

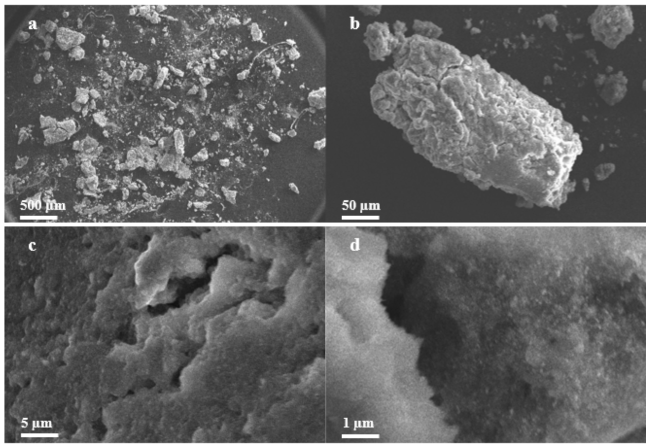


**Figure 6**. AgNPs morphological analysis using SEM (a) 30x magnification, (b) 500x magnification, (c) 5,000x magnification and (d) 10,000x magnification

The components detected in EDS in the synthesized AgNPs (Figure 7.) are carbon (C), oxygen (O), sodium (Na), magnesium (Mg), silicon (Si), phosphorus (P), sulfur (S), argon (Ar), potassium (K), calcium (Ca), copper (Cu), silver (Ag), cadmium (Cd). Based on


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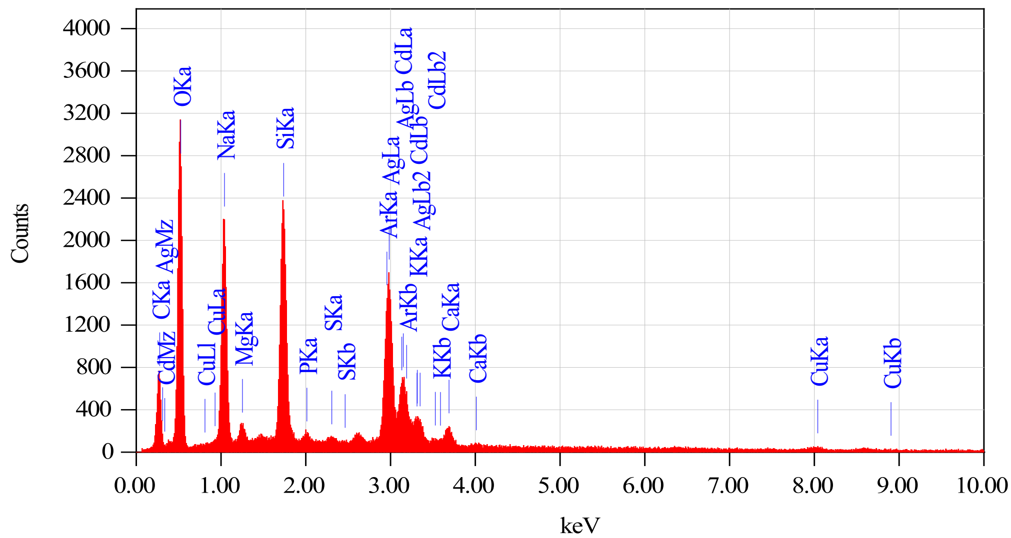
the elemental composition data of silver nanoparticles in Figure 7, it shows that the ratio of each element (K) of the Ag compound has a high ratio of 13.6014, so the biosynthetic results do indeed contain silver. In terms of mass percentage, it can be observed that the elements carbon (C) and oxygen (O) have a mass percentage of, namely; 15.92% and 44.53% and the percentage of silver mass (Ag) is 9.91%. These results refer to the biosynthetic process during the formation of nanoparticles, where the use of a water extract bioreductor of kecemcem leaves will affect the results of the elemental composition of AgNPs. Several other elements were also detected, which probably came from other secondary metabolites in kecemcem leaves. Residual elements arising from the tool used which was not cleaned completely or used several times with different samples, is detected by the EDS instrument

**a**




**b**

ZAF Method Standardless Quantitative Analysis

Fitting Coefficient : 0.1725

| Element | (keV) | Mass% | Sigma | Atom% | Compound | Mass% | Cation | K |
|---|---|---|---|---|---|---|---|---|
| C K | 0.277 | 15.92 | 0.14 | 25.57 | | | | 4.9744 |
| O K | 0.525 | 44.53 | 0.34 | 53.69 | | | | 46.5120 |
| Na K | 1.041 | 11.13 | 0.12 | 9.33 | | | | 10.8168 |
| Mg K | 1.253 | 0.79 | 0.04 | 0.63 | | | | 0.8626 |
| Si K | 1.739 | 7.53 | 0.08 | 5.17 | | | | 8.8772 |
| P K | 2.013 | 0.31 | 0.02 | 0.20 | | | | 0.4631 |
| S K | 2.307 | 0.17 | 0.01 | 0.10 | | | | 0.2657 |
| Ar K | 2.957 | 4.33 | 0.07 | 2.09 | | | | 5.9388 |
| K K | 3.312 | 0.58 | 0.04 | 0.28 | | | | 0.9433 |
| Ca K | 3.690 | 0.85 | 0.03 | 0.41 | | | | 1.3664 |
| Cu K | 8.040 | 0.65 | 0.06 | 0.20 | | | | 0.9093 |
| Ag L | 2.983 | 9.91 | 0.15 | 1.77 | | | | 13.6014 |
| Cd L | 3.132 | 3.30 | 0.10 | 0.57 | | | | 4.4690 |
| Total | | 100.00 | | 100.00 | | | | |

**Figure 7**. (a) Spectrum EDS and (b) elemental composition of silver nanoparticles.

Based on the research conducted, the particle size formed has an average size of 26.88 nm, a polydispersity index (PdI) of 0.597 (Table 1.) and a zeta potential value of AgNPs is -25.3 mV. A good PdI value indicates good long-term stability and a PdI value close to zero indicates a homogeneous or monodisperse particle size, while a PdI value that exceeds 0.5 to 1 indicates that the particles have a high degree of heterogeneity or polydisperse (Jalab *et al.*, 2021). These results indicate that the formed AgNPs colloidal particles have poor stability, while the negative value (-) in the results indicates that the formed AgNPs layer is negatively charged. Another study by Singh et al., (2018), zeta potential value of AgNPs was found to be -24.7mV, suggesting the presence of negative charges on the nanoparticle surface. Zeta potential value also indicates strong electrostatic repulsion between the synthesized AgNPs, confirming stable nanoparticles formation. According to (Helmlinger *et al.*, 2016), AgNPs has a small size, below 100nm.


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**Table 1**. AgNPs PSA Results

| Concentration | Replication | Z-average (nm) | (PdI) |
|---|---|---|---|
| Concentration 0,5% | I | 27.09 | 0.599 |
| | II | 26.78 | 0.591 |
| | III | 26.79 | 0.603 |
| **Average** | | 26.88 | 0.597 |

**AgNPs Analysis as a Tetracycline Biosensor**

The detection ability of AgNPs was performed by the addition of antibiotic tetracycline at a fixed concentration of 10 ppm solutions into AgNPs solution. The change of absorption intensity was evaluated using UV-vis spectroscopy. The noteworthy change in the intensity of the SPR band was clearly observed upon the addition of tetracycline and the color of the solution instantly (within one minute) changed from brownish red color to clear slightly reddish (Figure 8.), indicating the high selectivity of AgNPs toward the detection of tetracycline.

Based on the research, the AgNPs formed and analyzed on a UV-Vis spectrophotometer has a peak at a wavelength of 405 nm, whereas when AgNPs is mixed with tetracycline (MNT), the wavelength shifts to 366 nm (Figure 9.). This is due to a shift in wavelength to a shorter wavelength region (hypsochromic shift) due to substitution or solvent effects (Buncel & Rajagopal, 1990). This is in line with the research of (Kaur *et al*., 2022), there was a hypsochromic shift in the AgNPs peak from 415 nm to 390 nm when mixed with cephalexin. This was due to a hypsochomic jump, namely the blue shift, which is thought to be the peak for the formation of AgNPs bonds with cephalexin.

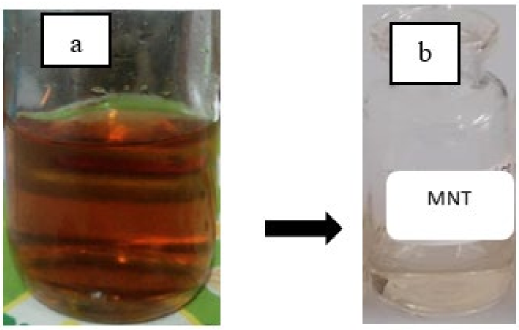


**Figure 8**. AgNPs (a) and MNT solution (b)

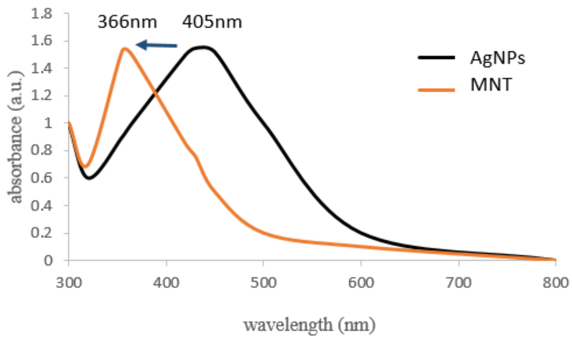


**Figure 9**. Comparison of AgNPs and MNT peaks.

Hipsochromic shift can occur because AgNPs binds to tetracycline causing AgNPs to be substituted in the tetracycline structure. This causes the conjugation structure of tetracycline to change into an organic compound that has a single bond (σ), thereby reducing the conjugation process in the tetracycline structure. This will absorb UV wavelengths in the direction of wavelengths between 200-400nm. The more AgNPs that binds to tetracycline or the number of tetracycline conjugate structures decreases, the wavelength will continue to shift below 200nm (ultraviolet vacuum). This is what causes a blue shift or hypsochromic in the interaction of AgNPs with tetracycline ((Kaur *et al*., 2022; Singh *et al*., 2023). Prediction of possible interactions between nanoparticles and tetracyclines as shown in Figure 10. In the recent research, the presence of the antibiotic kanamycin in milk samples can be detected using AgNPs. That suggesting negligible interference by the other components present in the milk, which makes the proposed method applicable for the direct determination of kanamycin in real samples (Singh *et al*., 2018). It is hoped that the AgNPs obtained from this research can be used to detect tetracycline in real samples.


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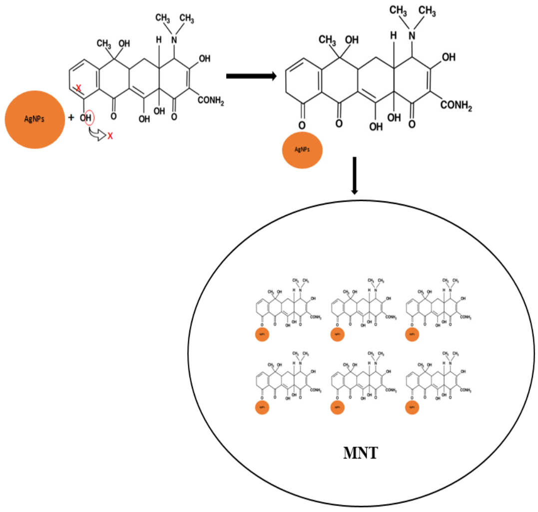


**Figure 10**. Predicted reaction mechanism of AgNPs with tetracycline.

**Validation of Analysis Methods**

Validation of the analytical method begins with linearity and concentration series which is carried out by measuring 10 MNT concentrations with a UV-Vis spectrophotometer at a wavelength range of 300-800 nm. The results of the linear regression equation obtained are y = 0.0142x + 0.1399 with a correlation coefficient (r) of 0.983. According to (Kadian *et al*., 2016) linear regression analysis has a minimum value of 0.98 to meet the requirements of good linearity, whereas according to (Araujo, 2009) the value indicates a correlation coefficient (r) which is close to 1, thus indicating a linear relationship between the measured absorbance value with the analyte concentration value (Kadian *et al.,* 2016; Araujo, 2009). Based on the research results, and compared with the literature, the data obtained has a linear correlation. The higher the sample concentration, the greater the absorbance.

The validation analysis of the LOD and LOQ methods was carried out by observing the absorbance obtained from the MNT regression curve, then calculating the MNT LOD and LOQ. Based on the calculations carried out, the LOD value is 0.8792 ppm and LOQ value was obtained at 2.930 ppm, this means that the sample concentration measurement obtained must be more than 2.930 ppm so that the measurement results can be said to be accurate. From the calculations made based on the precision absorbance data, the RSD result is 3.36%. Based on the results of the calculation of the precision value obtained, it is quite precise. This is in accordance with the range in the literature, where the precision of the RSD value is below 16%. Based on the (Bruce *et al*., 1998), the acceptable %RSD value for an analyte concentration of 1 ppm is 11% (Bruce *et al*., 1998). This result means that the method has closeness or suitability of the test results with the supposed value, so that it can be said that the method used is appropriate and precise. Based on the calculation results, the %recovery value obtained is 82.58%. Based on the analyte requirements, should have an acceptable %recovery value of 80-120% so it can be concluded that the method has good accuracy (FDA, 2001).

## 4. Conclusion

Water extract of kecemcem leaves can be used as a bioreductor in the synthesize of silver nanoparticles. AgNPs formed at a maximum wavelength of 405 nm has an absorbance of 3.627. Based on TEM, PSA, and SEM-EDS analysis, the morphology formed was spherical, with an average particle size of 26.886 nm with a zeta potential value of -25mV. In the characterization of AgNPs using FTIR, there are hydroxyl groups. Nanoparticles formed from the biosynthesis of kececem leaves can act as a tetracycline biosensor, in which a shift in wavelength occurs when AgNPs interacts with tetracycline. Synthesized AgNPs have been demonstrated as potential colorimetric sensors for the selective detection of tetracycline, respectively based on SPR. Based on the method validation carried out, it was obtained that linearity was in the form of a value (r) of 0.983, an LOD value of 0.879 ppm, and an LOQ of 2.93 ppm. The precision value obtained is 3.356%, and an accuracy of 82.58%.

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