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# EFFECT OF PLANT ORGANS OF *Ficus deltoidea* IN THE SYNTHESIS OF SILVER NANOPARTICLES

## (Kesan Organ Tumbuhan *Ficus deltoidea* dalam Mensintesis Nanopartikel Perak)

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**Abstract**

The effect of different plant organ extracts (leaf, stem, fig and root) of *Ficus deltoidea* var. *kunstleri* (King) Corner (Mas Cotek) in the synthesis of silver nanoparticles (AgNP) was studied. The plant analysis using liquid chromatography-mass spectrometry (LCMS) yielded more than 100 phytochemical compounds in each organ, of which around 50 % belong to the phenolics, flavonoids and polyphenols compounds. The biomarker (chemical marker) compounds (vitexin and isovitexin) were only found in the leaf, and Total Phenolic Content (TPC), as well as Total Flavonoid Content (TFC) were observed to be the highest in fig compared to other organs. The localized surface plasmon resonance (LSPR) of the biosynthesised AgNP using all organs was found at 409 to 428 nm. The capping and stabilization of AgNP by phytochemical compounds were verified by Fourier transform infrared (FTIR) spectroscopy as the vibration and stretching of -NH, $-CH_3$, $-CH_2$, -CH, C=O and -OH functional groups were found. There was a high abundance of phytochemical compounds in each organ: gallic acid, kaempferol-3-(6"-caffeoylglucoside), quercetin-3-O-rhamnoside and kaempferol 3-(3",4"-diacetylrhamnoside) in leaf, stem, fig and root, respectively. These compounds belong to the phenolics, flavonoids and polyphenols groups responsible for reducing $Ag^+$ to $Ag^0$ (AgNP). The results indicated that the fig which contained the highest TPC and TFC formed the highest LSPR peak for the formation of AgNP. The most important finding was the ability of each plant organ of *F. deltoidea* in the AgNP biosynthesis.

**Keywords**: plant organs, *Ficus deltoidea*, biosynthesis, nanoparticles

**Abstrak**

Kesan ekstrak organ tumbuhan yang berbeza (daun, batang, ara dan akar) dari pokok *Ficus deltoidea* var. *kunstleri* (King) Corner (Mas Cotek) dalam mensintesis nanopartikel perak (AgNP) dianalisis dalam kajian ini. Analisis pokok menggunakan spektometri jisim kromatografi cecair (LC-MS) menemui lebih dari 100 sebatian fitokimia di setiap organ, di mana sekitar 50% tergolong dalam sebatian fenolik, flavonoid dan polifenol. Sebatian penanda bio (kimia) (vitexin dan isovitexin) hanya terdapat pada daun, dan kandungan jumlah fenolik (TPC) serta kandungan jumlah flavonoid (TFC) diperhatikan pada bahagian ara adalah

tertinggi berbanding organ lain. Resonans plasmon permukaan setempat (LSPR) bagi AgNP biosintesis menggunakan semua organ didapati dalam lingkungan 409 hingga 428 nm. Pembatasan dan penstabilan AgNP oleh sebatian fitokimia disahkan oleh spektroskopi inframerah transformasi Fourier (FTIR) sebagai getaran dan perenggangan kumpulan berfungsi -NH, $-CH_3$, $-CH_2$, -CH, C=O dan -OH di kesan. Terdapat sebilangan besar bahan fitokimia di setiap organ iaitu galik asid, kaempferol-3-(6"-kafeglukosida), quersetin-3-O-ramnosida dan kaempferol 3-(3",4"-diasetilramnosida) di bahagian daun, batang, ara dan akar masing-masing. Sebatian ini tergolong dalam kumpulan fenolik, flavonoid dan polifenol yang bertanggungjawab untuk penurunan $Ag^+$ ke $Ag^0$ (AgNP). Hasil kajian menunjukkan bahagian ara yang mengandungi TPC dan TFC tertinggi membentuk puncak LSPR tertinggi untuk pembetukan AgNP. Penemuan yang paling penting adalah kemampuan setiap organ pokok *F. deltoidea* dalam biosintesis AgNP.

**Kata kunci**: organ tumbuhan, *Ficus deltoidea*, biosintesis, partikel-nano

## Introduction

Plant organs such as leaves, stems, roots, flowers, fruits, and seeds have diverse phytochemical compounds [1]. The type and concentration of the phytochemical compounds depend on the metabolism and the functions of the plant organs [2]. Diversity of the growing climate, different harvesting times and stress conditions affect the concentration of the phytochemical compound in the same species or source of the plant [3, 4, 5]. Phytochemical compounds are plant-part specific and vary extensively among different plant populations [5]. It usually consists of tannins, saponins, alkaloids, terpenoids, steroids, proteins, amino acids, phenolic and flavonoid compounds [6]. These phytochemicals give colour and odour for protection and reproduction, serve as phytoalexins for pathogen resistance, are involved in hormonal functions for growth and signalling, act as antifeedants or toxins for pests protection, and allelochemicals for defence against herbivorous animals [7].

Furthermore, plant phenolics and flavonoids can reduce $Ag^+$ to $Ag^0$ producing silver nanoparticles (AgNP) because of their functional groups being rich in electrons, forming complexes that are disunited to form AgNP. It is acknowledged that the hydroxyl group (O-H) of the phenolics and flavonoids firmly hold the $Ag^+$ in an oligomeric cluster, resulting in the formation of aggregates that stabilise into AgNP [8].

For many years, plants with high phytochemicals have been used as medicinal herbs due to their high nutritional values, rich sources of antioxidants that may slow down ageing processes, reduce inflammation and prevent certain diseases [1, 9]. One of the native medicinal plants available in Malaysia is *Ficus deltoidea* Jack var. *kunstleri* (King) Corner, which is also known as Mas Cotek. Traditionally, this plant has been developed as an alternative treatment for diabetes, hypertension, blood flow, gout, pneumonia, cardiac problems, diarrhoea and skin infection [10]. The chemical marker of this plant which are vitexin and isovitexin (flavonoid group) have many medicinal benefits to human. Previous studies have shown that these substances exhibit anti-tumour, anti-inflammatory, and antinociceptive properties [7, 8].

Typically, *F. deltoidea* can reach a height of 2 m, possesses whitish-grey stems and bark when mature. It has broadly spoon-shaped leaves, The leaves range in sizes from 4 to 8 cm with bright green colour on top and rust-red to olive-brown at the bottom. Furthermore, this plant produces yellow to orange figs with a width of about 1.0 to 1.5 cm [12] and also has fibrous brown roots. The different organs of *F. deltoidea* like the leaf, stem, fig and root may contain different phytochemical compounds. In the biosynthesis of AgNP, the reducing ability of any plant organ strongly depends on the presence of a phytochemical compound. However, there are still limited studies on the effect of plant organs on the synthesis of AgNP. This study focused on the analysis of phytochemical compounds of different organs of *F. deltoidea* and its initial effect on the formation of AgNP.

## Materials and Methods

### Chemical and reagents

Silver nitrate ($AgNO_3$) ≥ 99.0% was purchased from Vchem. Aluminum chloride ($AlCl_3$) ≥ 98.0%, sodium nitrite ($NaNO_2$) ≥ 99.0%, sodium hydroxide (NaOH) 99%, nitric acid ($HNO_3$) 69%, potassium bromide for infrared (IR) spectroscopy, methanol ≥ 99.8% for liquid chromatography usage, ethanol ≥ 99.9% for liquid chromatography usage, formic acid 97.5-98.5% and quercetin ≥ 95% were procured from Merck. Both vitexin ≥ 95.0% and isovitexin ≥ 98.0% were acquired from Sigma-Aldrich. Gallic acid was purchased from R&M Chemical.

### *F. deltoidea* plant extract

The *F. deltoidea,* with the age of about four years, was provided by the Department of Agriculture Malaysia, Pontian, Johor Branch. The plant was segregated according to its organs: leaf, stem, fig and root. They were cleaned with deionised water to remove unwanted impurities like sand, gravel, insects, bugs, and other contaminants. After that, the plant organs were dried in a 60 °C oven. The dried plant organs were then ground and sieved with a mesh size of 0.45 µm. The dried powdered biomasses were stored in a chiller at 4 °C for later use. Figure 1 shows the illustration of the process flow to obtain the dried plant biomasses.

The process involved mixing 2 g of the dried biomass with 100 ml of boiled deionised water. A hotplate with a magnetic stirrer was used to stir the mixture for 30 minutes at 100 °C [13]. The plant extracts were then filtered with Whatman No 1 filter paper, and the filtrate was stored at 4°C for future use.

### Screening of Phytochemical Compounds using Liquid Chromatography Mass Spectrometer (LC-MS)

Ethanol 96% was used as a maceration solvent to macerate 45 g of dried biomass. This procedure took three days to complete. The maceration solvent was replaced for every 24 hours. The ratio of the dried biomass powder weight (g) to solvent (ethanol 96%) volume (L) was 500:6. The maceration solution was collected, and an evaporator was used to separate the solvent and dissolved chemicals generated from the dried biomass. The evaporation procedure was used to generate a paste of approximately 45 g, which was then considered to be at 100% concentration. The LCMS analysis was performed using the Shimadzu LCMS 2010 EV and a reverse phase LC system. The mobile phase of acetonitrile distillation to water ratio was 7:3, and the ammonium acetate concentration was 10 mmol/L. The LC pump gradient system with a vacuum pump flow velocity of 1 mL/min and C-18 column (150 mm × 2.1 mm) with a column temperature of 40 °C were used in this analysis. Only 10 µL of the sample was injected in a single run using a gas nitrogen generator at 250 °C. Positive Ion Electroscopy was used with a Mass Spectrometry Detector (SPD-10 AVP).

### Analysis of Vitexin and Isovitexin using High-Performance Liquid Chromatography (HPLC)

Vitexin and isovitexin were selected as chemical markers for F. deltoidea varietal identification [10]. A round bottom flask was filled with 5.0 g of dried biomass and added with 80 mL of ethanol 96%. The sample was allowed to cool after being refluxed for 30 minutes. Thereafter, the sample was filtered with Whatman No. 1 filter paper and the filtrate was evaporated. Methanol (HPLC grade) was used to dissolve 50.0 mg of the dried extracts. It was then injected into the High-Performance Liquid Chromatography (HPLC) column after being filtered with a 0.45 µm syringe filter using HPLC Agilent 1260 Infinity. The injection volume was 10 µl, and the isocratic mobile phase that carried the sample to be separated was a mixture of six volumes of 1% formic acid and four volumes of methanol. The flow rate was set to 1.0 mL/min and the sample was separated in a C-18 column with 250 mm × 4.6 mm and an oven temperature of 30 °C. For each sample, the run time was 15 minutes.

### Total Phenolic Content and Total Flavonoid Content

Total phenolic content (TPC) was determined by mixing 1.0 mL of the sample with 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of $Na_2CO_3$ 20% solution. The mixture was then incubated for half an hour at 40

°C before being measured with a Jenway 7200 Visible spectrophotometer at 750 nm. For Total Flavonoid Content (TFC) analysis, 0.3 mL $NaNO_2$ solution 5% was mixed with 1 mL sample and the mixture was left for five minutes. After that 0.3 mL of $AlCl_3$ 10% solution was added to the sample and the solution was makeup to 10 mL. The sample was measured using the visible spectrophotometer at 510 nm. TPC and TFC values were calculated based on the formula (Equation 1 and 2) obtained from the literature [13], which were expressed in mg of Gallic acid per 1g dry extract (Gallic Acid Equivalent; GAE) and mg of Quercetin equivalent (QE) per gram of dry mass (g), respectively.

$$\text{Total phenolic compound, (GAE)} \left(\frac{\text{mg}}{\text{g}}\right), = \frac{C_1 V}{M} \quad (1)$$

$$\text{Total Flavonoid Content, (QE)} \left(\frac{\text{mg}}{\text{g}}\right), = \frac{C_1 V}{M} \quad (2)$$

where, GAE is gallic acid equivalent, QE is quercetin equivalent, $C_1$ is concentration gained from calibration curve, V is volume of extract, mL and M is mass of plant biomass (g).

**Formation of AgNP using Organs of *F. deltoidea***

For the formation of the AgNP, the aqueous plant extracts were added to 10 ml of $AgNO_3$ 1 mM according to the synthesis conditions as shown in Table 1. The synthesis parameters were selected based on our experiment on optimizing the AgNP synthesis method (Data not shown and will be published elsewhere). The formation of AgNP was monitored using a Jenway 7200 Visible Spectrophotometer in the visible range of 350 to 750 nm. This is due to the fact that AgNP exhibited a localized Surface Plasmon Resonance (LSPR) band at this range based on its free electron excitation [14]. The functional groups of *F. deltoidea* extracts and biosynthesised AgNP (powder form) were analyzed using Fourier Transform Infrared Spectroscopy (FTIR). This analysis was performed on a Perkin Elmer FTIR 1600 using the KBr technique in the range of 4000 – 400 $cm^{-1}$.


Figure 1. Illustration of the process flow of preparing dried plant biomasses of different organ

## Results and Discussion

The LCMS results in Figure 2 indicated that the leaf, stem, fig, and root produced signals at different retention times (RT) of 127, 129, 104, and 119 peaks, respectively. The RT signals could be attributed to the presence of phytochemical compounds. Based on the chromatogram, it can be said that more than 50% of them were phenolics, flavonoids and polyphenol compounds. Among the phytochemical compounds found in *F. deltoidea* are rutin, quercetin, naringenin, vitexin, and isovitexin. The selected phytochemical

compounds content (in percentage) is shown in Table 2.

Notably, these phytochemical compounds act as biological defenses for unicellular and multicellular plants, protect biological cells, tissues and organs of plants from damages caused by oxidative stress, and as plant metabolites, they prevent inflammation and promote carbohydrate metabolism [14-19]. The highest phytochemical compounds identified in each organ are tabulated in Table 2. On that note, gallic acid was identified in the leaf, kaempferol-3-(6"-caffeoylglucoside) in the stem, quercetin-3-O-rhamnoside in fig and kaempferol 3-(3",4"-diacetylrhamnoside) in the root. The molecular structures of these phytochemical compounds are shown in Figure 3.

To validate the chromatogram, the retention time (RT) of a specific flavonoid of vitexin and isovitexin at 7.4 and 8.7 minutes, respectively was used in the HPLC analysis (Figure 4). Notably, these compounds were found at 17.84 ± 2.57 ppm of vitexin and 13.92 ± 0.44 ppm of isovitexin in the leaves of *F. deltoidea*. It was abundantly found in the leaf organ. This finding is in line with the study that has been conducted by Abdullah et al. [17], which found that the concentration of vitexin was higher than that of isovitexin in the leaf of *F. deltoidea*. He at al. [18] mentioned that in *Lemna minor* plant, vitexin was found to be converted to isovitexin and not in reverse direction. These phytochemical compounds serve as siderophores, attractants and feeding deterrents in plant [17]. From previous studies, these compounds also found in various plants such as mung beans, wheat leaves and bamboo [18].

Figure 5 shows the TPC and TFC values of the extracts of *F. deltoidea* measured using the Folin-Ciocalteu method and Aluminium chloride colourimetric assay, respectively. It is important to determine both compounds composition in the plants as the formation of AgNP highly depends on their presence. This was emphasised by Asmat-Campos et al. as they stated that the presence of polyphenol promotes the reduction and capping of nanoparticles [19]. In the Folin-Ciocalteu method, the reagent used was composed of a mixture of phosphotungstic acid and phosphomolybdic acid, which were reduced to a mixture of blue oxides of tungsten and molybdenum once the phenols were oxidised. The blue colour produced had a maximum absorption in the 750 nm range and was proportionate to the total amount of phenolic compounds present in the sample [20]. To determine the TFC, the Aluminium chloride reacted with the flavone, flavanol and hydroxyl/dihydroxyl group in the flavonoids [21]. From the result, the leaf organ of *F. deltoidea* contains 28.56 ± 0.87 (GAE) mg/g TPC and 761.32 ± 13.00 (QE) mg/g. These results are lower than the leaf of *Vigna Unguiculate* Dhawala which contains 508.7 (GAE) mg/g and 1303.1 (QE) mg/g, respectively [22]. Meanwhile, the fig organ of *F. deltoidea* had the highest TPC and TFC values compared to other organs. Even though the number of phytochemical compounds in fig is the lowest, this organ has the highest TPC and TFC values.

Table 1. AgNP synthesizing conditions

| Plant Organs | Plant Extract Volume (mL) | Reaction Time (Hours) | Reaction Temperature (°C) | pH of Plant Extract |
|---|---|---|---|---|
| Leaf | 1.0 | 30 | 60 | 10 |
| Stem | 1.0 | 21 | 90 | 12 |
| Fig | 0.8 | 33 | 100 | 10 |
| Root | 3 | 21 | 90 | 12 |

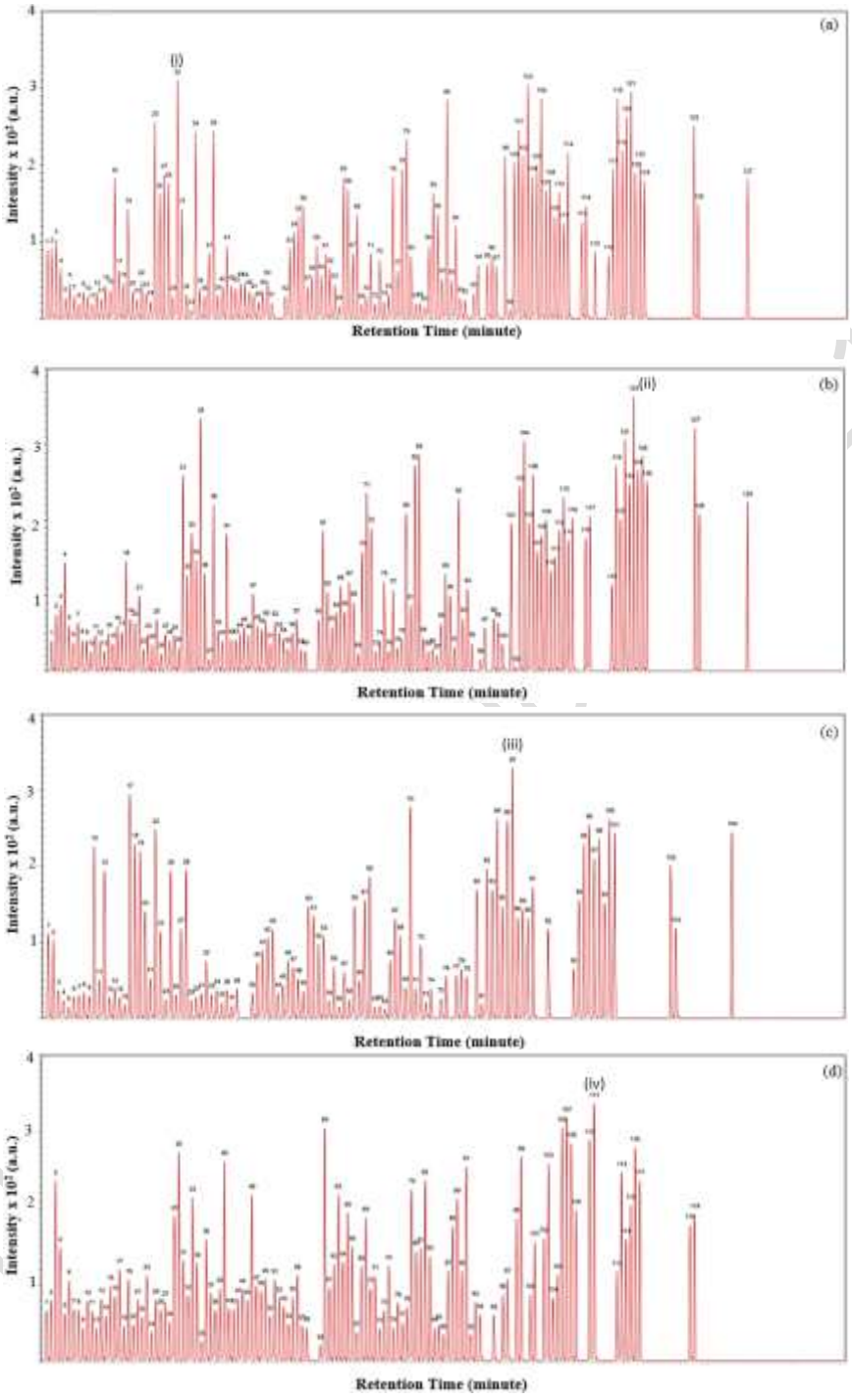


Figure 2. LCMS chromatogram of the phytochemical compounds found in (a) leaf, (b) stem, (c) fig and (d) root of *F. deltoidea*. The labelled peaks are the highest phytochemical compounds contained in each organ which are (i) gallic acid was identified in the leaf, (ii) kaempferol-3-(6"-caffeoylglucoside) in the stem, (iii) quercetin-3-O-rhamnoside in fig and (iv) kaempferol 3-(3",4"- diacetylrhamnoside) in the root.

Table 2. The percentage of phytochemical compounds in *F. deltoidea*

| Phytochemical Compounds | Retention Time (min) | Mass to Ion Ratio (m/z) | % Compound | | | |
|---|---|---|---|---|---|---|
| | | | Leaf | Stem | Fig | Root |
| *P*-coumaric acid | 1.859 | 164.05 | 1.91 | 1.79 | 2.71 | 1.28 |
| Gallic acid[i] | 3.042 | 170.02 | 2.32 | 2.32 | 2.28 | 1.45 |
| Chlorogenic acid | 12.421 | 354.10 | 2.12 | 1.58 | 2.55 | 1.72 |
| Luteolin-7-glucoside | 22.628 | 448.10 | 2.15 | 1.80 | 2.41 | 1.05 |
| Quercetin-3-O-rhamnoside[iii] | 24.003 | 432.11 | 2.14 | 1.39 | 3.02 | 1.75 |
| Kaempferol 3-(3"-acetylrhamnoside) | 24.77 | 75.03 | 1.23 | 1.60 | 1.21 | 2.15 |
| Kaempferol 3-(3",4"-diacetylrhamnoside)[iv] | 28.208 | 516.13 | 1.10 | 1.42 | 1.08 | 2.28 |
| Kaempferol-3-(6"-caffeoylglucoside)[ii] | 35.508 | 610.13 | 2.21 | 2.52 | 2.17 | 1.89 |

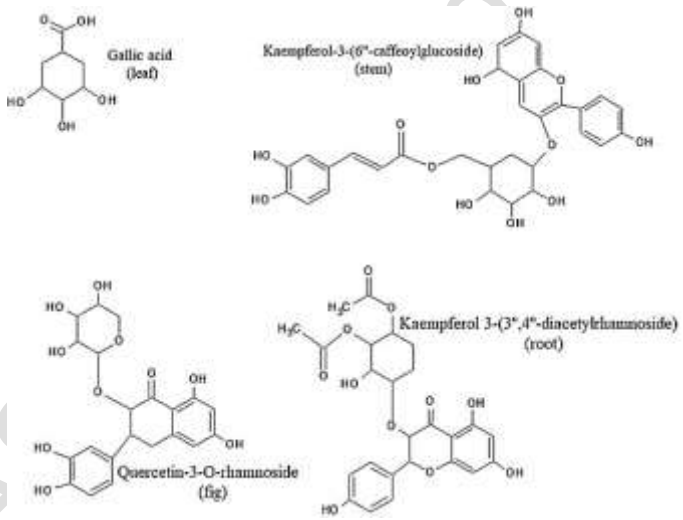


Figure 3. Molecular structures of main phytochemicals compounds in each *F. deltoidea* organs

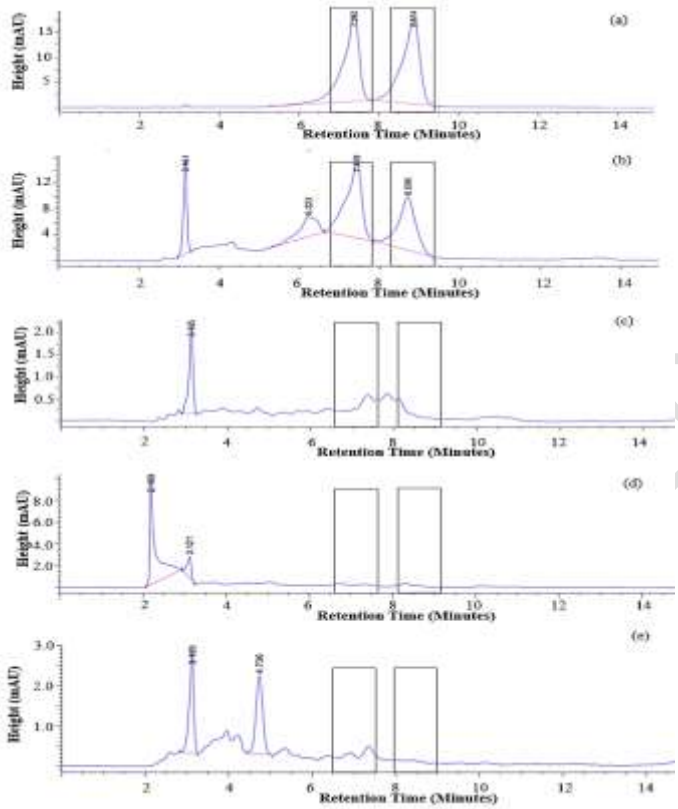


Figure 4. HPLC chromatograms of vitexin and isovitexin in (a) mixed standards, (b) leaf, (c) stem, (d) fig and (e) root

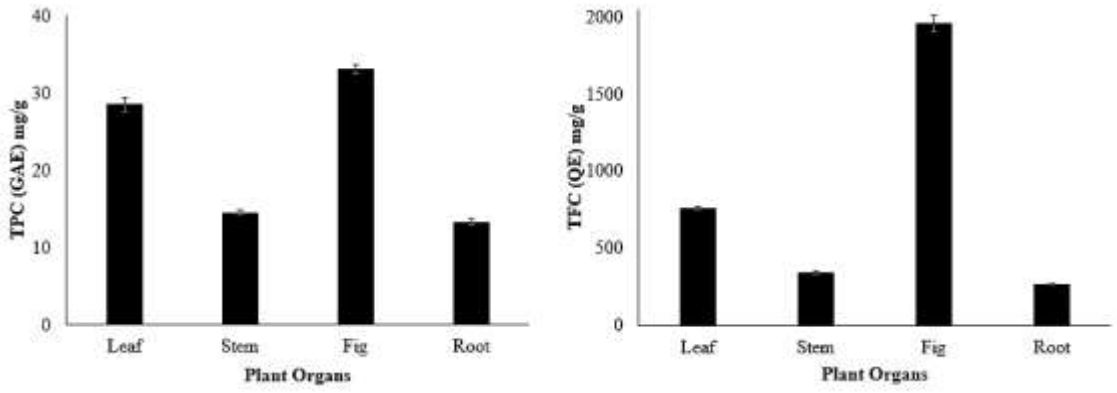


Figure 5. TPC and TFC values of different plant organs of *F. deltoidea*

In this study, different organs of the plant extract were used in the biosynthesis of AgNP. As shown in Figure 6(a), the formation of AgNP was conducted by monitoring the LSPR peak in the range of 350 to 750 nm. The FTIR spectra of the plant biomass and the biosynthesised AgNP using each plant organ can be seen in Figure 6(b and c).

In Figure 6(a), the LSPR absorbance peak of the biosynthesised AgNP using *F. deltoidea* was observed in the visible region between 409 and 428 nm. Based on the LCMS result, the stem had the highest number of phytochemical compounds while fig recorded the lowest percentages; however, the TPC, TFC and capability to form AgNP according to LSPR peak did not follow this trend. This means that the phenolics, flavonoids and polyphenol compound greatly influenced the ability to synthesis AgNP compared to the number of phytochemical compounds in the plant organ. According to Beer-Lambert's Law, it can be deduced that the formation of AgNP was highest when using the fig organ. This aligns with the works of Lavola et al. [5], where they discovered that phenolic content in the fruit of *Empetrum hermaphroditum* was the highest compared to other organs. Nonetheless, the role of each organ in plant physiology could be attributed to the presence of different phenolics content [23]. This also leads to different organ capabilities in the biosynthesis of AgNP. Moreover, the complexity and massive amounts of different phytochemicals in any plant extract is the major challenge in determining the exact phytochemical responsible for the biosynthesis of AgNP [22, 23]. Taking this into account, it is safe to suggest that the oligomeric cluster may comprise one or more phytochemicals that capped and stabilised the AgNP [26].

In the FTIR spectra of *F. deltoidea* plant extract, the peak at 3430 $cm^{-1}$ revealed stretching of the amide (N–H) bond and hydroxyl (–OH) groups. From the IR spectra, the absorption peak at 2920 $cm^{-1}$ could be attributed to $–CH_3$ and $–CH_2$ stretching vibrations. C-H stretching from the alkane group causes the absorption peak at 2830 $cm^{-1}$. The C=O stretching had a peak at 1735 $cm^{-1}$, whereas C=C stretching had a peak at 1620 $cm^{-1}$. The O-H bending from the carboxylic acid functional group is represented by the peak at 1430 $cm^{-1}$. All of these functional groups corroborated the phytochemical compounds identified by LCMS. The same stretching and vibration bands were observed in the biosynthesised AgNP, indicating the presence of phytochemical compounds from *F. deltoidea* in the AgNP samples, which acted as a capping and stabilising agent in Figure 6(b and c).

From the plant analysis, *F. deltoidea* can be interpreted as a herbal plant that is rich in phenolics, flavonoids and polyphenols compounds. Its organs (leaf, stem, fig and root) have unique functions and metabolisms that make the amount and variety of phytochemical compounds differ from one another. Hence, they have the ability and capability of synthesising AgNP. It can be expected that the AgNP produced has distinct characteristics. The high TPC and TFC values impacted the formation of AgNP tremendously. This was reflected by the fact that the plant organ (fig) that had the highest phenolics and flavonoids exhibited the highest LSPR peak. This composition contributed to the highest formation of biosynthesis AgNP by the fig organ. In this study, phytochemical compounds are not only used as a reducing agent but also proved to be the stabilising agent for AgNP.

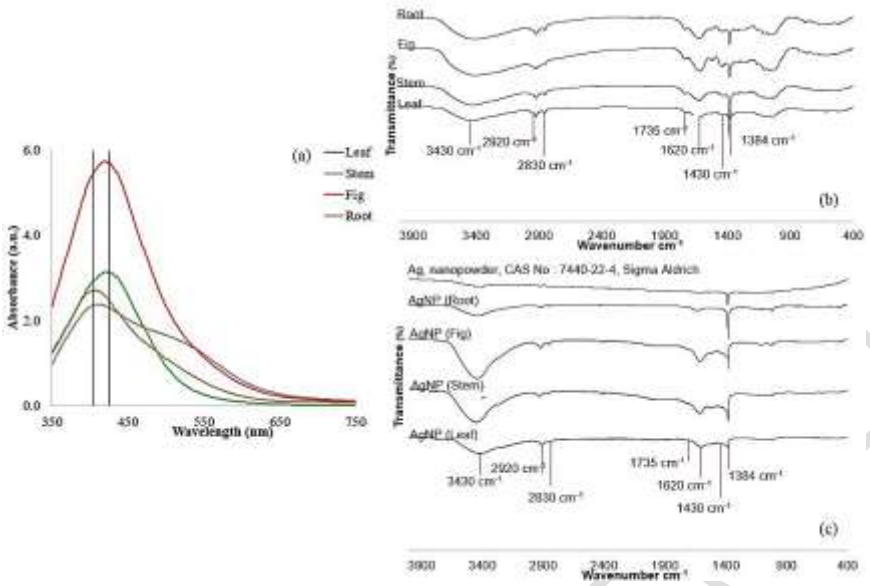


Figure 6. (a) LSPR absorbance for AgNP, (b) FTIR spectra of plant biomasses (b) and (c) biosynthesised AgNP

## Conclusion

*F. deltoidea* Jack var *kunstleri* (King) Corner is a native herb that is rich in phytochemical compounds. From the LC-MS analysis, more than half of the phytochemicals belong to the phenolics, flavonoids and polyphenols group. The validation analysis also revealed that a specific flavonoid of vitexin and isovitexin were only detected in the leaf organ. The phytochemical compounds in plant extracts have the capability in the biosynthesis of AgNP by assisting the LSPR absorbance peak formed at 409 to 428 nm. The FTIR result revealed that the phytochemical compounds also served as a capping and stabilising agent for the formation of AgNP due to the diverse composition and high content of phytochemical compounds in *F. deltoidea*. In conclusion, this study adds to the body of knowledge on phytochemical compounds in plant organs spectrum in biosynthesising AgNP. Thus, this information could be used to manufacture AgNP from suitable plant organs.

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