Phytosynthesis of Iron Nanoparticle from Averrhoa Bilimbi Linn.

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Abstract. This paper demonstrates iron nanoparticles (FeNP) was synthesized from natural sources of Averrhoa bilimbi Linn. The plant extracts act as natural reducing agent in producing FeNP. There is no addition of any surfactants during the nanoparticles formation. Gravimetric analysis is used to calculate the percentage yield of plant extracts. TPC and DPPH assay method were used to evaluate antioxidant activity in different A. bilimbi extracts and synthesized FeNP. Based on the analyses, it showed that fruit has the highest percentage yield and antioxidant activity followed by leaf, twig and bark. Analysis from TPC, fruit contains 27.26 mg GAE/g and 39.46 mg GAE/g for FeNP. DPPH assay showed fruit extract has the highest free radical antioxidant activity with 61.93% in A. bilimbi and 80.00% in FeNP. Phytosynthesis of FeNP were examine by using UV-Vis spectrophotometer. Based on the spectra, it showed that FeNP recorded peak absorbance at 465 nm, 450 nm, 460 nm and 440 nm for UAE-F, UAE-L, UAE-T and UAE-B, respectively. FTIR analysis shows the presence of strong alcoholic bond, aldehyde, stretch amine and alkene that was responsible in reduction process to form FeNP. The result of UV-Vis and FTIR showed that the existance of FeNP and involvement of functional group that were responsible on the formation of nanoparticles.

1. Introduction
Nanotechnology is one of the fastest developing research in this area of study caused by the improvement of their characteristics and properties [8]. Generally, nanotechnology able to measure ultrafine particles with their ranging size from 1-100 nanometers [14]. The researchers also have been progressively observed the shape and size of nanoparticles depends on the physiochemical properties by using advanced techniques [14].

Recently, many studies have proven that the plant extracts act as a potential precursor for the synthesis of the nanoparticles in non-hazardous ways [4]. The plants are used successfully in the synthesis of several greener nanoparticles such as cobalt, copper, silver, gold, palladium, platinum, zinc oxide and iron oxide [8]. During past decade, it has been demonstrated that many biological systems, plants able to transform inorganic metal ions into metal nanoparticles by the reductive capacities of the proteins and metabolites present in the extracts.
Synthesis using biological method is compatible with the green chemistry principles. Besides, biological method for synthesizing nanoparticles also makes use of environmental friendly, non-toxic and safe reagents. Nanoparticles synthesized using biological techniques or green technology have diverse natures, with greater stability and appropriate dimensions since they are synthesized using a one-step procedure [1].

2. Experimental works

2.1. Chemicals
Gallic acid, DPPH solution, Sodium carbonate, Folin-Ciocalteu reagent, Iron (II) Sulfate Heptahydrate, Iron(III) Chloride Hexahydrate.

2.2. Plant Materials
Fruits, leaves, twig and bark of A. bilimbi samples were obtained from Kg. Alor Ibus, Kedah. The A. bilimbi part of the plants which is fruits, leaves, twig and bark washed cleanly and dried in the oven. After that, samples were grinded into powder forms by using grinder and transfer into the beaker.

3. Methodology

3.1 Preparation of Plant Extract
The methods to prepare plant extract were followed Zulhaimi et. al. [17]. For the plant parts, bark was added along with fruits, leaves, twig and bark. Cold maceration (CM) and ultrasonic-assisted extraction (UAE) were utilized. Distilled water was used as solvent.

3.2 Percentage Yield of Plant Extract
In order to analyse the percentage plant yield extract, the extracts of evaporated dried extracts based on dry weight basis were calculated by using gravimetric analysis as the following equation:

\[
\text{Yield (g/100 g of dry plant)} = \frac{(W_1 \times 100)}{W_2}
\]

Where \( W_1 \) is the weight of the extract after the solvent evaporation and \( W_2 \) is the weight of the initial powder used to extract.

3.3 Total Phenolic Content (TPC Assay)
1 mL extract solutions of different plant parts were added in the test tube following 1 mL of Folin-Ciocauteau reagent were added and mixed thoroughly. The mixtures were dismissed for 5 minutes at room temperature. Sequently, 4 mL of sodium carbonate (75 g/L) and 10 mL of distilled water were added and thoroughly mixed. The mixture were idle at room temperature for 2 hours. Standard curve was obtained using addition of 0.5 mL Gallic acid solution in extraction sample and the absorbance with UV-Visible Spectrophotometer at 765 nm was determined. total phenolic content was calculated using the following equation:

\[
\text{TPC} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard x Dilution factor}} \times \text{Shake x Weight of extract}
\]

3.4 DPPH Assay
In this assay, purple radical DPPH was reduced by antioxidant samples to the corresponding pale yellow hydrazine. 0.5 mL of DPPH (25 g/L) solution was added to 1 mL of extraction sample solution (with different solvents). Mixtures were shake vigorously and kept at room temperature for 30 minutes in dark. Then, the absorbance determined using calibrated UV-Visible spectrometer at 517 nm. This scavenging activity was calculated as % inhibition using the formula:

\[
\text{DPPH scavenging activity} = \left(\frac{\text{Absorbance control}}{\text{Absorbance sample}}\right) \times 100
\]
3.5 **Synthesis of FeNP**

FeNP using *A. bilimbi* was synthesized using Iron (III) Chloride Hexahydrate (FeCl$_3$.6H$_2$O), Iron (II) Sulfate Heptahydrate (FeSO$_4$.7H$_2$O) and plant extract. FeCl$_3$.6H$_2$O and FeSO$_4$.7H$_2$O were prepared and mixed together in a beaker with a ratio 2:1 by using magnetic stirrer. 5 mL of prepared plant extract was added to mix for 1 hour. The formation of extract colour to dark green or black colour observed indicating the formation of FeNP [6]. The samples were tested using UV-Vis Spectrophotometer and FTIR.

3.6 **UV-Vis Spectrophotometer**

The blank and sample solutions were put in a Quartz cuvette and the baseline corrected using plant extracts as the blank. The spectra of FeNP obtained in the wavelength range of 400 – 600 nm.

3.7 **Fourier Transform Infrared (FTIR)**

Fourier Transform Infrared (FTIR) spectrometry is a complex method of spectroscopy with the ability to identify materials and determine the quality of a sample. FTIR spectroscopy was performed to determine the functional groups of *A. bilimbi* that appeared in FeNP. The functional groups that present in the synthesized nanoparticles were found at a region of 650 cm$^{-1}$ to 4000 cm$^{-1}$.

4. **Results and discussion**

4.1 **Percentage Yield of *A. bilimbi* Extracts**

Figure 1 showed the percentage yield of CM and UAE using water as solvent for each parts of *A. bilimbi*. The percentage yields of CM for fruit (CM-F), leaf (CM-L), twig (CM-T), and bark (CM-B) by using water as solvent were 49.77%, 29.88%, 24.93% and 19.95%, respectively. The percentage yields for UAE for fruit (UAE-F), leaf (UAE-L), twig (UAE-W), and bark (UAE-B) by using water solvent were 49.87%, 22.25%, 20.40% and 19.92%, respectively. From the graph, UAE-F showed highest percentage of plant extract compared to the others plant parts. The result also give the same to CM-F.

Percentage yield of extract of CM-F and UAE-F was higher than other parts, showed that fruits powder has high solubility than the other parts [5]. Moreover, the preliminary phytochemical studies by revealed the presence of carbohydrates, proteins, amino acids, flavonoids, tannins, bitter principles, essential oil, valepotriates, coumarin, and terpenes in *A. bilimbi* fruits [2].

![Figure 1. The percentage yields of CM and UAE of *A. bilimbi*.](image-url)
4.2 Total Phenolic Content assay (TPC assay)

The Figure 2 showed TPC of FeNP on different plant parts of A. bilimbi. UAE method was preceded due to simpler process yet produced high percentage yield of extract. Based on figure in 4.2, TPC for FeNP from UAE process for all plant parts has higher value in comparison to the plant extract, with 32.22 mg GAE/g for UAE-FeNP-F, 27.04 mg GAE/g for UAE-FeNP-L, 7.12 mg GAE/g for UAE-FeNP-T and 4.27 mg GAE/g for UAE-FeNP-B. It was noticed that TPC value of plant extract is lower with 19.26 mg GAE/g for UAE-F, 12.26 mg GAE/g for UAE-F, 12.26 mg GAE/g for UAE-L, 9.11 mg GAE/g for UAE-T and 2.17 mg GAE/g for UAE-B.

![Figure 2](image)

**Figure 2** The total phenolic content of FeNP and A. bilimbi extracts.

The phenolic compound such as phenolic acids, flavonoids and tannins are considered to be major influenced to the ability as antioxidant plants. The higher content of total phenolic content in A. bilimbi extract facilitates the reduction of iron ions to nano scale sized of iron particles [14]. The nano size of FeNP resulted to higher total phenolic compounds value [14].

4.3 DPPH assay

Figure 3 shows the result of DPPH scavenging activity of A. bilimbi on different plant parts. The DPPH activity result of UAE-F, UAE-L, UAE-T and UAE-B were expressed in percentage which is 61.93%, 43.54%, 25.12% and 11.00%, respectively. Meanwhile, the result of DPPH activity of FeNP using plant extracts also compared in Figure 4.3. It was found that DPPH value is drastically high, with 80.00% for UAE-FeNP-F, 53.75% for UAE-FeNP-L, 31.5% for UAE-FeNP-T and 17.78% for UAE-FeNP-B.

DPPH (1,1-diphenyl-2-picrylhydrazyl) was one of the free radical scavenging activity that use to measure antioxidant activity. This is the simplest and most widely reported method for screening antioxidant activity in foods and plants [9]. Besides, DPPH scavenging activity of antioxidants is due to their hydrogen donating ability. Therefore, there is correlation reduction between the numbers of DPPH molecules with the number of hydroxyl groups [9]. Furthermore, the radical scavenging activities of FeNP also appear to be regenerative making it more effective and allowing scavenging of multiple free radical by antioxidant nanoparticles [9]. Considering the high surface area to volume ratio, it appears that FeNP, showed higher tendency to interact to reduce DPPH free electron [9].
4.4 Phytosynthesis of FeNP
The reduction of iron ions and formation of FeNP occurred instantly after mixing of ferric chloride and ferric sulphate with plant extracts. This can be examined from the colour changing upon addition of each plant parts. It was observed that the solution turned the colour from yellow to brownish for fruit. Meanwhile, other plant parts for leaf, twig and bark were all turned into black, which indicates the formation of FeNP.

4.5 UV-Vis Spectrophotometer
UV-Vis Spectrophotometer was carried out to confirm the formation of FeNP. Light sources will emit over ultraviolet and visible light spectra [7]. The samples solutions of transition metals tend to have distinct colour characteristics with certain corresponding peaks. Previous studies suggested FeNP that formed from plant extract showed a band peaking in between the range of 400 nm to 600 nm. The spectra clearly depicted maximum absorption peak which indicated the formation of FeNP. The Figure 4 showed the peaks for UAE-FeNP-F, UAE-FeNP-L, UAE-FeNP-T and UAE-FeNP B.
From the result in Figure 4, it was observed that the most broad absorption peak was found at the range of 440 and 465 nm. This could be due to the excitation of nanoparticles from ground state to the excited state [12]. For FeNP-UAE-F, broad peak absorbance was recorded at 465 nm. For FeNP-UAE-L, it was observed that the peak is at 450 nm, which evidenced that phytochemical content extract act as reducing agent to metal precursor to the formation of nanoparticle.

Meanwhile, the most broad absorbance peak at 460 nm and 440 nm were recorded for twig and bark. All of the most broad absorbance peaks were shown at different wavelength for different type of reducing agent. This may occur due to agglomeration and settling of nanoparticles in a cuvette which shown different broad peak in each result.

4.6 Fourier Transform Infrared (FTIR)
The Figure 5 interpreted the FTIR spectra of synthesized FeNP from (a) UAE-F and (b) UAE-L. The absorption peaks in FTIR spectra of nanoparticles for fruit were located mainly at 3384 cm$^{-1}$ and 1640 cm$^{-1}$. Meanwhile, Figure 4.6 depicted the FTIR spectra of FeNP using (a) UAE-T and (b) UAE-B. The Figure 4.9 (a) showed peak at 3368 cm$^{-1}$ and 1644 cm$^{-1}$. The Figure 4.6 (b) showed peak at 3383 cm$^{-1}$ and 1639 cm$^{-1}$. The peak at 3384, 3368 cm$^{-1}$ are due to strong alcoholic bond like aramanthine and phenolic compound presence in the aqueous phase. The peak can also be found in Sathya et al. that studied on iron oxide nanoparticles [18]. 1641, 1644 and 1639 cm$^{-1}$ indicate the presence of (C=O) bond that were accountable for the formation of FeNP.

![Figure 5. The FTIR spectra of FeNP synthesis using (a) UAE-F and (b) UAE-L.](image)

The representative peaks in FTIR spectra of FeNP for fruit, leaves, twig and bark have similar characteristic stretching vibration as shown in Figure 5 and Figure 6. The band at same range of absorption where the functional group was corresponded indicated the involvement of amine (N-H) at band 3366, 3384, 3368 and 3383 cm$^{-1}$. There may have possibility that amine group played an important role in the reduction of metal ion. Besides, band 1640, 1641 and 1644 cm$^{-1}$ indicate the presence of alkene group (C=C) stretching frequency that may responsible in reduction process [7]. It was noted that the spectra shared same type of stretch vibration with variable type of intensity [7].
5. Conclusion
The green approach of preparation FeNP are able to be produced by using different parts of *A. bilimbi*. The *A. bilimbi* is very suitable as natural reducing agent to produce FeNP. CM and UAE technique were carried out to extract *A. bilimbi* plant parts which are fruit, leaves, twig and bark. Water was used as the solvent. The antioxidant activity were examined by using total phenolic content (TPC) assay and DPPH assay showed fruit has the highest antioxidant activity. Based on the two techniques, it showed UAE gave highest result for antioxidant activity. UAE were then chosen to synthesize FeNP. FeNP that formed from ferric chloride and plant extract were analysed by using UV-Vis Spectrophotometer and FTIR spectra analysis. The result showed that the existance of FeNP and possibility of functional group that were responsible in the reduction process of FeNP.

Acknowledgement
Authors would like to thank Universiti Malaysia Perlis, School of Bioprocess Engineering, Universiti Malaysia Perlis and Institute of Nano Electronic Engineering, Universiti Malaysia Perlis for the support of laboratory works. This study was funded by Universiti Malaysia Perlis under STG 9001-00554.

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