CHAPTER 3

MATERIALS AND METHODS

3.0 CHAPTER OVERVIEW

This chapter provides the information about the materials and methods which were used in this study.The plant extract was used to synthesize the nanoparticles and the characterizations of their physico-chemical properties were studied using different analytical techniques such as UV-Vis spectrophotometer, FESEM, EDX, XRD, FT-IR, particles size analyzer and zeta potential. Also, the biological properties of the synthesized nanoparticles such as antibacterial and antioxidant activities were carried out in this study. Besides, the *in-vitro* anticancer activity against HCT-116 colon cancer cells was evaluted. The *in-vitro* anticancer study was evaluated by mRNA gene expression using the qRT-PCR technique. Furthermore, the morphological characterization and flow cytometry study were also carried out.

3.1 PLANT COLLECTION AND IDENTIFICATION

Commelina nudiflora is an edible weed plant and it has a variety of medicinal importances for human. It is available in many countries mainly Malaysia, Indonesia, India and other tropical regions such as Burma, Vietnam and eastern parts of China. The whole healthy plant *C. nudiflora* (*rumput tahi itek*) was procured from Maran, Pahang state, Malaysia in March 2012. Figure 3.1 shows processed *C. nudiflora* plant materials in dry and powder form. The plant was selected for this study on the basis of its availability, medicinal property and cost-effectiveness. The plant was taxonomically authenticated by Associate Professor. Dr. Norazian Mohammad Hassan, Kulliyyah of

Pharmacy, International Islamic University, Malaysia. The voucher specimen was deposited at Kulliyyah of Pharmacy.

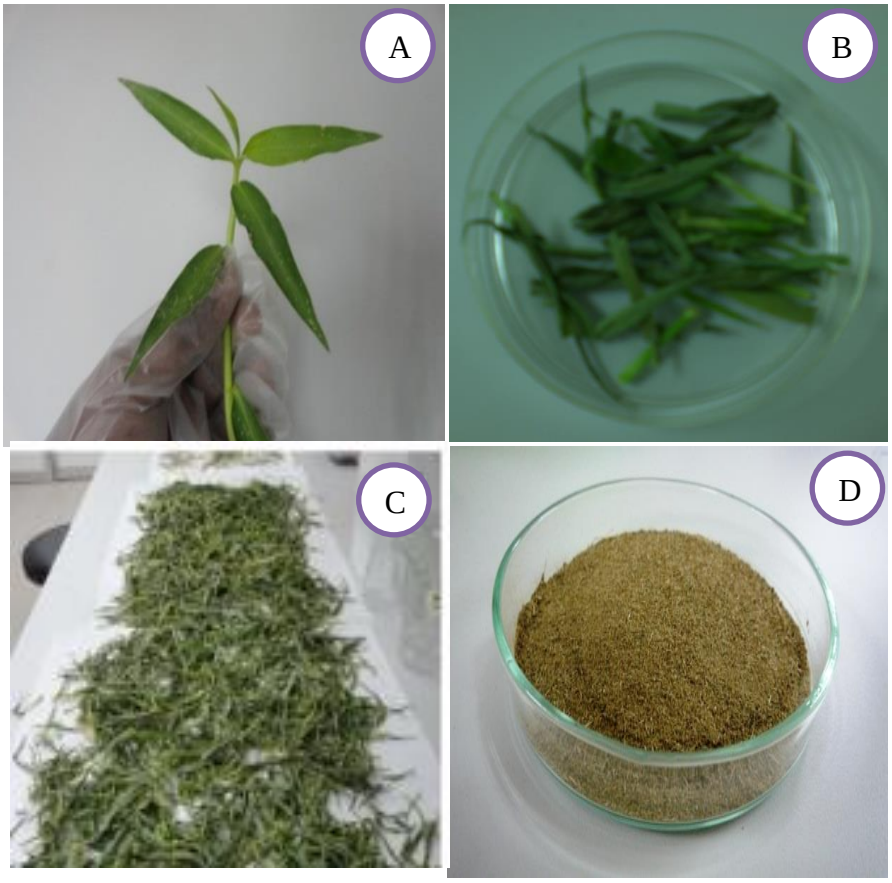

Figure 3.1: Photographs of *Commelina nudiflora* plant a) whole *C. nudiflora* plant b) cleaned with tap water c) dried in shadow condition d) Powder form of *C.nudiflora*

3.1.1 Chemicals and glasswares

Chloroauric acid ($HAuCl_4$), silver nitrate ($AgNO_3$), sodium chloride (NaCl), ascorbic acid, autylated hydroxyanisole (BHA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) were purchased from Sigma-Aldrich Chemicals, (USA). Other solvents and chemicals were of analytical grade and used throughout the experiments without further purification. All experiments reagents were prepared using Milli-Q (MQ) water unless stated otherwise. All glasswares were rinsed twice with de-ionized water.

3.1.2 Plant extract preparation

Fresh and healthy whole plants were rinsed thoroughly with running tap water followed by distilled water to remove all the dust and unwanted debris, then cut into small pieces and used for extract (broth) preparation.

Then, 10 g of finely incised plants were weighed separately and added with 100 ml distilled water in a 250 ml flat bottom flask. The mixture was boiled for about 25 minutes at 65 °C. The extracts were filtered through Whatman No. 1 filter paper, and the procured clear solutions were stored in a refrigerator at 4°C, until further use. All reactions were conducted in sterile conditions to maintain the effectiveness and accuracy of the reaction.

3.2 BIOSYNTHESIS OF METALLIC NANOPARTICLES

3.2.1 Biosynthesis of gold nanoparticles

An amount of 20 ml of *C. nudiflora* aqueous extract was added with freshly prepared aqueous solution of 80 ml of $HAuCl_4 \cdot 3H_2O$ (10^{-4} M) and briskly stirred for 10 minutes in a reflex system at 37 °C (Figure 3.2). The color formation indicates that the gold ions were reduced into nano-gold in 30 minutes at room temperature. The formation of pale yellow into pink colour shows the nanoparticles formed in the solution. The experiment was optimized with molar solution of gold precursor and the plant extracts to achieve the different morphologies of nanoparticles (Narayanan and Sakthivel, 2008, and Philip, 2009).

3.2.2 Biosynthesis of silver nanoparticles

The aqueous plant extract of 30 ml was added with 170 ml aqueous solution of $AgNO_3$ (10^{-4} M) and stirred for 30 minutes. Reduction took place gradually and it was completed in 30 minutes as shown by the pale yellow colour that changed into stable light brown colour of the solution, which shows Ag^+ ions were completely converted