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Research Article

PHYSICOCHEMICAL PROPERTIES OF *POLYGONUM MINUS* NANOPARTICLE TREATMENTED BY HYDROTHERMAL METHODS

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ABSTRACT
In this study, *Polygonum minus* nanoparticle were prepared by using planetary ball mill associated with hydrothermal process to improve its size reduction. Particle size analysis employing photon correlation spectroscopy was carried out to record the effect of hydrothermal treatment on the particle size produce. The functional groups and polar compounds were determined using Fourier Transform Infra-Red (FTIR), while Thermogravimetric Analysis (TGA) has been used to analyze the thermal stability of *Polygonum minus* nanoparticles prepared by ball milling assisted hydrothermal process. The result showed that after hydrothermal treatment, the average size of particles was produced in 329nm which was significantly smaller compare without treatment. FTIR results showed the peak at region of 1,600-1,500 cm^{-1} is due to the stretching vibration of carbonyl group and 1300-1,000 cm^{-1} indicate a stretching of the C-H group. Sample ball milling with hydrothermal (treated) showed higher Total Phenolic Content (TPC) (1186.12 mgGAE/g of dw) as well as Total Flavonoid Content (TFC) (102.5 mgQE/g dw) whereas lowest value shown by sample ball milled without treatment with 897.23 mgGAE/g and 72.40 mgQE/g dw respectively. These results showed that the ball milling treated with hydrothermal method had a potential to produce the kesum nanoparticles with a smaller size and better antioxidant properties.

Keywords: *Nanoparticle, Polygonum Minus, Planetary Ball Mill, Hydrothermal*

INTRODUCTION
Polygonum minus is commonly known as Kesum in Malay have unique taste and flavour which belongs to the family Polygonaceae and primarily grown in temperature region. It grows wild in damp areas near the river banks, ditches and lakes. This plant believed to have originated from Southeast Asia countries such as Malaysia, Thailand, Indonesia and Vietnam (Qader *et al.*, 2012). *P. minus* is a plant having a kind of nice, sweet and pleasant aroma. The plant leaves is popularly used as an ingredient in Malaysian food such as laksa (spicy noodle dish), kerabu (fried herbal rice), asam pedas (spicy tamarind curry) and tom yam (spicy tangy soup) (Vimala *et al.*, 2003). In the Malaysian traditional medicine application it is used to treat headache, digestive disorders, stomach problem, reduce the dandruff problem and pain (Vimala *et al.*, 2003; Nurain *et al.*, 2012; Qader *et al.*, 2011). In terms of food commodity in Malaysia, kesum is considered an important plant in Malaysia because of the domestic market and also for export. National Agrofood Policy (NAP), 2011 – 2020, kesum is included in eleven of the herbs that will be focused by the Malaysian Government.

In a previous study Nurain *et al.*, (2012); Qader *et al.*, (2011); Vimala *et al.*, (2012); Maizura *et al.*, (2011); Vimala *et al.*, (1999) showed that kesum had the highest antioxidant activity among a various of Malaysian herbals that have been screened. Due to its high volatile oil constituents contain high phenolic compounds (Qader *et al.*, 2011) such as gallic acid, rutin, coumaric, flavonoids such as myricetin and quercetin (Qader *et al*., 2012), alkaloids, tannins and terpenoids (Imelda *et al.*, 2014), it has a potential application in perfumes and powerful antioxidant as well as antibacterial activity. Numerous reported showed that plant with major constituents such as flavonoids and lignans are hard to be effectively absorbed primarily due to poor solubility either in oil or water, complex chemical structure and large particle size and surface (Nurain *et al.*, 2012; Vimala *et al.*, 2012; Qader *et al.*, 2011). The application of

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nanotechnology promised many advantages, such as control particle size and surface properties (Mohanraj and Chen, 2006), increasing compound solubility and improving the absorbency of plant medicines compared with the respective crude plant preparations (Bhadoriya *et al.,* 2011). Therefore, numerous studies have been conducted to improve the value in use and overcome this problem. It has been practically proved that particle size reduction strategies, particularly nanotechnology can improve the solubility and increase the dissolution rate of poorly water soluble active pharmaceutical ingredients (Hidayah *et al.,* 2012; Sonada *et al.*, 2008; Kim *et al.*, 2008; Su *et al.*, 2006; Itoh *et al.*, 2003).

On the other hand, hydrothermal technique is one of the most important tools for advanced materials in recent year. It has advantages in the processing of nanostructure materials in a wide variety of technological applications such as electronics, optoelectronics, catalysis, ceramics, magnetic data storage, biomedical and biopotonics which is helping in processing monodispersed and highly homogeneous nanoparticles (Byrappa and Adschiri, 2007). Thus, in this research the ball milling assisted with hydrothermal will be used to get herb nanoparticle. The ball milling is a common method in the size reduction of material. The process will be produce a nanoparticles through the impact forces is generated by the action of centrifugal forces while the processing in hydrothermal will help to get a highly homogeneous nanoparticle. The physical and chemical properties of *P. minus* as affected by the size reduction using planetary ball milling assisted with hydrothermal process also will be studied.

MATERIAL AND METHODS

Sample Preparation

Polygonum minus (kesum) was purchased from a local wet market in Shah Alam, Selangor. The plant material was washed with running tap water and rinsed with distilled water to remove dirt and contaminants. The clean plant material was stored in an oven at 50°C and was left to dry for 3 days and weighed until get a constant weight. The dried plant material was ground using a conventional rotor mixer. The ground powder was then sieved through a 100µm sieve and stored at 4°C prior to further process.

Preparation of Nanoparticle by Ball Milling

The grinding system name planetary ball mill (Retsch PM 200) was chosen to produce a kesum nanoparticle due to its simplicity. A Kesum powder 0.3 gram was first mixed with water in 50 cm^3 stainless steel grinding jar containing 25 grams of protective jacket of zirconium. Zirconium oxide balls of 2 mm were used to utilized for wet millings respectively. The ratio of kesum powder to water used in this study was kept at 0.1:10. The milling time was set for 4 hours at 550 rpm which was the optimum ball-milled time for kesum nano suspension to show the smallest particle size according to our previous finding. All the samples produced were collected and kept in the airtight container prior to further analysis.

Hydrothermal Treatment

The kesum nano suspension from ball milling was treated using a modification hydrothermal process. In this method kesum ball milled was put into 50 ml Teflon-lined stainless-steel autoclave. After that the sample was treated at 100^0C at 1 hour. This parameter was chosen based from preliminary results show at this condition the size reduction of kesum has a significant decreased. The kesum nano suspension after treatment was then collected and some of the sample is freeze dried for analysis.

Particles Analysis

The particle size distributions were measured by means of a laser sizer (Malvern Mastersizer S, UK) based on light diffraction at 25°C and the reading was taken at 173° scattering angle. Prior to measurement, the nanosuspension of each sample were taken out and adjust to 5% (w/v) concentration and introduced into a disposable cuvette to measured in triplicate. The functional groups and carbon bonding of kesum nano particle were analyzed using FT-IR/FT-NIR Spectrometer (Perkin Elmer Spectrum 400). Spectra were obtained at a resolution of 1 cm^{-1} in the range of 4000 cm^{-1} to 400 cm^{-1} with accumulation of 32 scans. The spectrum, then was plotted and major peaks identified using Origin Pro8 computer software.

Total Phenolic Content (TPC) and the Total Lavonoid Content (TFC)

The antioxidant activity was estimated using the Total Phenolic Content (TPC) and the Total lavonoid Content (TFC). The total phenolic content was determined using the Folin– Ciocalteu reagent with

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analytical grade gallic acid as a standard. 300 µl of extract or standard solutions (0–500 ppm) was added to 7.9 ml deionized water and 0.5 ml of Folin–Ciocalteu phenol reagents. After 5 minutes, 2.0 ml of 20% sodium carbonate was added to the mixture and kept in dark for 2 hours.
The absorbance was taken at 750 nm by using a UV/VIS spectrophotometer (UV–VIS Helios Zeta, Thermo Scientific, USA) and expressed as mg of gallic acid equivalents per g of dry weight (mgGAE/g) (Su *et al*., 2006).
Meanwhile, total flavonoid content was determined by an aluminium chloride colorimetric method with some modification (Kim *et al.*, 2012). The absorbance was measured against distilled water as the blank at 510 nm and expressed as quercetin equivalents per gram of dry weight (mgQE/g dw).

RESULT AND DISCUSSION

Particles Size Analysis

The average size of the nanoparticles for *Polygonum minus* untreated and treated with 100ºC was determined at 354 nm for untreated and slightly reduced to 329 nm for treated 100ºC as shown in Figure 1. The ground nanoparticle sizes varied from 50 nm to 3000 nm with the 295 nm being the highest size amount with intensity 8.0% in untreated and 7.4% in treated sample.
The figure also indicates that the represents of mixture of smaller and larger particles present in the sample (Dynamic light scattering). The size distribution of nanoparticles can be understood by referring to the polydispersity index (PdI) which is the PdI value is in the range 0.428 to 0.402 for untreated and treated which is indicated in a medium range.

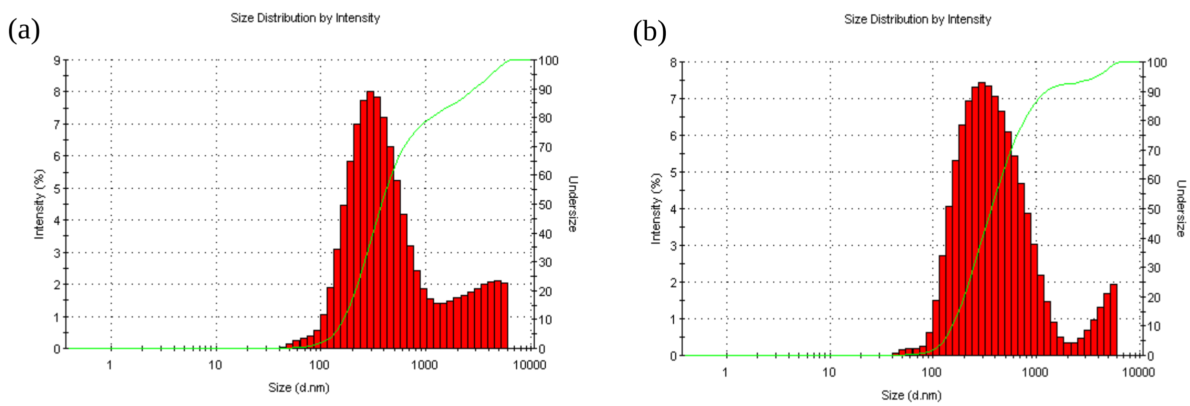

Figure 1: Particle Size Analysis of *P. Minus* Nanoparticle (a) Untreated and (b) Treated with Hydrothermal

This medium in PdI value also indicates the presence of larger particle after milling since the plant material is hard to grind below 100 nm (Ma *et al.*, 2009; Su *et al.*, 2006) due to the fibrous structure of plant material and limits the size reduction of particle. While the result in treated also indicates that the hydrothermal treatment can cause profound the delocalization or disrupting of plant cell wall (Lee *et al.*, 2008).

Fourier-Transform Infrared (FT-IR) Spectroscopy

The FT-IR spectra of the *Polygonum minus* (kesum) without ball mill and hydrothermal treatment (control), untreated and treated process with corresponding assignment of bands are shown in Figure 2. Previous reported mention that *Polygonum minus* chemical compositions, mainly consist of aldehydes and terpenes (Khairudin *et al.*, 2013; Baharum *et al.*, 2010).
Thus, FTIR spectroscopy is an accurate, fast and simple method to determine the functional group and polar compounds of the *Polygonum minus* sample.

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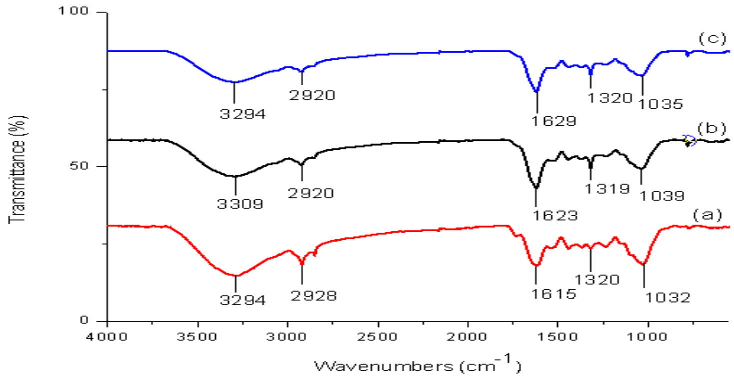

Figure 2: FTIR Spectra of *P. Minus* (Kesum) (a) Without Ball Mill and Hydrothermal (b) Untreated (c) Treated

All samples showed high transmittance at the wavenumber region 3,500 - 3,000 cm^{-1}, 2,900 – 2,800 cm^{-1}, 1,600 – 1,500 cm^{-1} and 1,300 – 1,000 cm^{-1}. According to Pavia and Kriz (2001), peak at wavenumber 3,500 – 3,000 cm^{-1} corresponds to hydroxyl absorption. The 2,900 – 2,800 cm^{-1} absorption peak corresponds to methylene C-H asymmetric stretching vibration. The peak at region 1,600 -1,500 cm^{-1} corresponds due to the stretching vibration of carbonyl group. While many C-O groups have peaks at 1,300 – 1,000 cm^{-1} and above generally this peak is the vibration peak of C-O in the alcohol hydroxyl group. The FT-IR also indicate that the sharp absorption peak at 1600 – 1,500 cm $^{-1}$ are assigned to C=O stretching vibration in carbonyl compounds which may be characterized by the presence of high content of terpenoids and flavanoids in the complex mixture of *Polygonum minus*. The presence of a narrow and sharp peak at ~2,928 cm^{-1} and ~2,920 cm^{-1} was assigned to C-H and C-H (methoxy compounds) stretching vibration respectively.

Thermo-Gravimetry Analysis (TGA)

Structural, functional group differences and size reduction in sample influence the thermal behavior and effect the transition temperature. TGA curves obtained from Figure 3 for *Polygonum. minus* (kesum) without ball mill and hydrothermal treatment (control), untreated and treated were not significantly different, indicating that the pyrolysis characteristics of samples were quite similar.

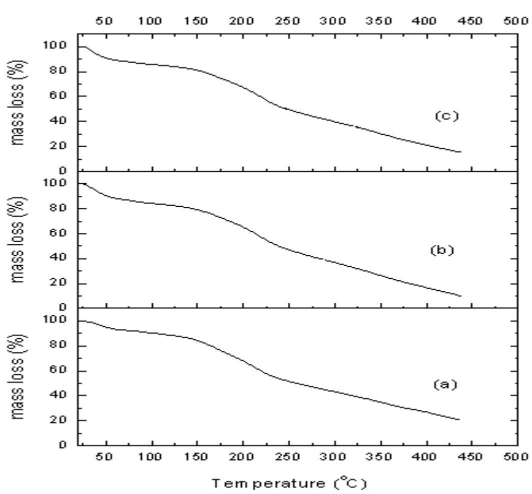

Figure 3: TGA Curves of *Polygonum Minus* Nanoparticle (a) Without Ball Mill and Hydrothermal Treatment (b) Untreated (c) Treated with Hydrothermal

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Almost all tested samples showed a two-stage obvious weight loss peaks below 300^0C. The early minor (below 100^oC) weight loss in samples is attributed to the loss of absorbed and structural water (Vendruscolo *et al.*, 2009; Bothara and Singh, 2012) or due to desorption of moisture as hydrogen bound water to the saccharide structure, with about 10% of the total mass loss. The second weight loss happened at a temperature range at 200 to 500^oC accompanying the softening and decomposition of a large number of cellulose, hemicelluloses and partial of lignin (Gao *et al.,* 2012; Zohuriaan and Shokrolahi, 2004) with about 55% of all tested samples.

From the result, it was found that the thermal stability of sample without ball mill and hydrothermal treatment show higher stability compare untreated and treated samples. Sample without ball mill and hydrothermal treatment decomposed at 245^0C, whereas the decomposition of untreated sample was 230^0C and 235^0C for treated sample.

This composition temperature was not much different in all samples because cellulose and starch derivative can stand to high temperature. Therefore, the ball milling and hydrothermal treatment do not much affect to the sample thermal stability.

Total Phenolic Content (TPC) and the Total Lavonoid Content (TFC)

The phenolic compounds are compounds released by plants which are formed in response to the reactive oxygen species (ROS) due to stress and are known to possess antioxidant activities (Gil *et al.*, 2000) and its play the key role as primary antioxidants or free radical scavengers. Due to their redox properties, the antioxidant activity of the phenolic compound, can take part as a role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa, 1994). The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase and scavenge free radical (Mallavadhani *et al.,* 2006; Lin *et al.*, 2005).

Polygonum minus nanoparticles significantly ($p<0.05$) influenced by ball mill and hydrothermal process. The TPC increased up to 34% due to treatment with hydrothermal which is the highest value shown by sample ball milled with hydrothermal (BMH) (1186.12 mgGAE/g extract) followed by ball milled (BM) (897.23 mgGAE/g) and *Polygonum* minus without ball milled treated with hydrothermal.Whereas, the TFC was in the ranged of 72.70 to 102.50 mgQE/g which is the *Polygonum minus* with ball milled treated with hydrothermal shown the highest TFC (102.50 mgQE/g of extract), followed by sample ball milled and without ball milled treated with hydrothermal (78.70, 72.70 mgQE/g of extract respectively).

The highest TPC and TFC values were shown in the *Polygonum minus* nanoparticles is probably due to particle size produced and it is proven that particle size reduction strategies using nanotechnology can improve the solubility and increase the dissolution rate of poorly water soluble active ingredients.

Conclusion

In this study, the grinding process using planetary ball mill could effectively reduce the particle sizes of *Polygonum minus* suspension and successfully prepared to nano scale. Our finding showed that the treatment using a hydrothermal process showed not significantly different in term of size and thermal properties. While, the FT-IR spectra revealed that some functional group is not clearly detect in *Polygonum minus* (kesum) without ball mill and hydrothermal treatment (control) due to polarization properties in different functional group.

On the other hand, untreated and treated process showed many functional group appear indicate the presence of carbohydrate, aldehydes and terpenes compound due to the solubility of functional group in water effect to milling and hydrothermal process. Highest TPC, TFC which leads to significantly ($p<0.05$) greater antioxidant estimation shown by *Polygonum minus* ball milled treated with hydrothermal (BMH) followed by ball milled (BM).

Thus, it can be conclude that ball milled treated with hydrothermal process can be applied to enhance the antioxidant properties of plant material.

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