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Ultrasonic Assisted Extraction on Phenolic and Flavonoid Content from *Phyllanthus niruri* Plant

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Abstract

Objectives: To find out the effect of ultrasonic assisted extraction with various solvents of phenolic and flavonoid compound extracted from *P. niruri*. **Methods/Statistical Analysis:** Phenolic content from *P. niruri* was analyzed using Singleton's method while the flavonoid content was analyzed by aluminum chloride colorimetric assay. **Findings:** The polyphenols extraction was greatly affected by the solvent type and concentration, particle size of the plant powder, solid liquid ratio and frequency. The highest phenolic content and flavonoid content were obtained at the solid ratio of 2g dry weight *P. niruri* powder at 119.28 mg GAE/g DW and 75.86 mg QE/g DW. **Application/Improvements:** The phenolic and flavonoid content reached its optimum extraction yield at the particle size of 125 µm, using 40% EtOH as solvent and the extraction yield increased when the ultrasonic frequency is increased to 53 kHz. The extraction method used in this work may serve as a useful guide to obtain optimum polyphenol extraction from *P. niruri.*

Keywords: Phyllanthus niruri, Total Phenolic Content, Total Flavonoid Content, Ultrasonic Assisted Extraction

1. Introduction

Phyllanthusniruri ver. name Dukunganak (Family: Phyllanthaceae) is a herb that grows up to 50 cm tall and having a smooth bark on the ascending branches with small flowers and tiny fruits filled with seed. It grows mainly in the tropical areas, thrives in wet rainforest conditions and spreads rapidly throughout the tropical and subtropical countries, including Malaysia, Indonesia, Thailand, Nigeria, Brazil, Philippine and India. Traditionally, *P. niruri* was used as a home remedy in many countries due to its well-known curative properties. In India, *P. niruri* is a common herb used to heal problems related to stomach, genitourinary system, liver, kidney and spleen. *P. niruri* extracts is known to restrict the growth of hepatitis B virus found in the blood stream, having antifungal, anti-viral and hypoglycaemic action which is useful for treatment of liver disease[1]. *P. niruri*has a diuretic property and hence often used in urinary tract infections and bacterial infections like cystitis and protastitis[2]. All the reported medical benefit from *P. niruri* is attributed to the polyphenols content from the plant material that must be extracted successfully before it can be used.

The yield and recovery of the bioactive components from plant materials is often affected by the extraction method used. In[1] performed a soxhlet extraction of *P. niruri*, using the extraction time of 3 hours to obtain 150 ml of extract. Meanwhile, in[2] employed a maceration extraction technique which consumes about 10 hours. Both the maceration and soxhlet extraction were the conventional and traditional method which normally requires higher temperatures and longer duration to obtain the extract. Furthermore, higher temperature that is often used in conventional extraction process may cause a thermal degradation of the polyphenol[3]. In addition, thermal intensive processes consume more energy and thus are not sustainable. Alternatively, a non-thermal intensive process such as the Ultrasonic Assisted Extraction (UAE)

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method was never been used to extract polyphenol from *P. Niruri* and hence this is the aim of this work.

The yield of bioactive component extraction from the plant materials depends on the mass transfer process involving the solvent (liquid) transport to the inner part of the plant materials (solid), the solubility of the solute and release of solutes from the solid matrix to the external bulk phase of the plant. With the aid of UAE, reduction of the mass transfer limitation for both internal and external transport can be achieved. Moreover, with the aid of the ultrasonic wave, cell membrane of the plant may break facilitating release of polyphenols to the bulk liquid. Therefore, UAE was selected for this research study.

Extraction is affected by the type of solvent used. In the extraction process, solvent will diffuse into the plant material and solubilize compounds with similar polarity[4]. Most of the previous work on *P. niruri*'s extraction used either water or methanol as solvent[5–7]. Although methanol facilitates extraction of polyphenols from *P. niruri*, they are not fit for purpose if the end product is used for pharmaceutical purpose. Hence, in this work only the Food and Drug Administration (FDA) approved chemicals i.e. ethanol, isopropyl alcohol and water were used as the solvent in the extraction process. In addition, no previous work on the effect of particle size on the yield of polyphenols extraction from *P. niruri*. Thus, the effect of different particle size i.e. <125 µm, 125 µm, 630 µm and 830 µm was evaluated in this work. It is known that the ultrasonic frequency affects the yield of polyphenols extraction, but no previous study in this regard for *P. niruri*. Therefore, the effect of sonication frequency was assessed at two different frequency setting i.e. 35 kHz and 53 kHz.

2. Materials and Methods

2.1 Chemicals and Plant Material

Sodium nitrite, ethanol, isopropanol, sodium hydroxide and Folin–Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). Aluminum hexachloride was obtained from Sigma Aldrich (St. Louis, MO). *P. Niruri* similar to that with a voucher specimen deposited in the Herbarium of RimbaIlmu, Institute of Science Biology, University of Malaya, Kuala Lumpur (voucher number KLU46618) were purchased from Malaysia Herbal Shop, Selangor, Malaysia. The dried plant was crushed into powder. The powder was kept in an air-tight plastic bag in a desiccator at room temperature to prevent moisture absorption prior to experiment.

2.2 Ultrasonic Assisted Extraction

The powdered plant material was weighed and mixed with 100 ml of solvent in a 250 ml sealed Erlenmeyer flask. UAE was carried out in a double frequency desk-top ultrasonic cleaner (Model: JK-DUCH-6210LHC) filled with 10.5 litres of water at 53 kHz and 270 W for 60 min and temperature was set at 50°C to study the effect of solvent type. Various solvent at different concentration ranging from 20% to 100% Isopropanol (IPA) or Ethanol (EtOH) mixed with ultrapure water were used for the extraction process. A suitable solvent that enabled a simultaneous extraction of both phenolic acid and flavonoid were chosen for the remainder of this work. The UAE supernatant was then separated from the residue by filtration using 0.45 µm nylon membrane filter.

2.3 Total Phenolic Content

Total Phenolic Content (TPC) was assessed using Singleton's method[8]. A sample aliquot of 0.125 ml was added to a centrifuge tube containing 0.5 ml of ultrapure water and 0.125 ml of the Folin–Ciocalteu reagent. 1.25 ml of 7% Na_2CO_3 solution was added after 3 minutes and the final volume was made up to 3 ml with ultrapure water. The solution was mixed well and incubated for 60 min in the dark. The absorbance was measured against the prepared blank reagent at λ = 760 nm using a calibrated ultraviolet–visible spectroscopy (Varian Cary 50). TPC of the leaves was expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW) by comparing with the calibration curve for gallic acid.

2.4 Total Flavonoid Content

Total Flavonoid Content (TFC) was measured using the aluminum chloride colorimetric assay[9]. A sample aliquot (0.2 ml) was added to a 15 ml centrifuge tube containing ultrapure water (4.8 ml). $NaNO_2$ (0.3 ml, 5%) was then added and mixed using a vortex mixer for 5 min. Subsequently, $AlCl_3$ (0.3 ml, 10%) was added, followed by addition of NaOH solution (2 ml, 1 M) and the total volume adjusted with ultrapure water to the final volume of 10 ml. The solution was mixed well and absorbance measured against a blank reagent at λ = 414 nm using a calibrated ultraviolet–visible spectroscopy (Varian Cary

50). The TFC of the sample solution was expressed as mg quercetin equivalents per gram dry weight (mg QE/g DW) by comparing with the calibration curve for quercetin.

3. Results and Discussion

3.1 Effect of Particle Size of *P. niruri* on Phenolic Compound Extraction

The particle size is one of the factors that affect the efficiency of the polyphenol extraction[10,11]. Therefore, the effect of particle size was first studied to determine the best particle size for the remainder of this work. The effect of particle size on the TPC and TFC is presented in Figure 1. The result clearly showed that the highest yield of TFC and TPC was obtained using the smallest particle size i.e. 125 µm and <125 µm. theoretically, the smallest particle has the highest surface area and hence the highest extraction yield. However, in this work the very fine particle (<125 µm) tends to float on the surface of the solvent, rendering the extraction process ineffective. Therefore, the plant matrix with the particle size of 125 µm was chosen for the remainder of this work since it has the highest extraction yield.

3.2 Effect of Solvent Type on Phenolic Compound Extraction

Total phenolic and flavonoid content assay is a common method to estimate the relative amount if phenolic compounds presence in the plant extracts, owing to their ability to enable a fast screening on the effect of extraction parameter to the relative extraction yield. According to[12], the phenolic compounds in the extract undergo a complex redox reaction with phosphotungstic and phosphomolybdiv acids from the Folin and Ciocalteu's reagent. Oxidations of the Folin and Ciocalteu's reagent by the respective number of the phenolic group cause a colour change in the sample. Figures 2 and 3 show that the highest phenolic content (44.55 mg GAE/g DW) and flavonoid content (61.99 mg QE/gDW) were obtained from the extract with aqueous 40% isopropanol solvent. A comparable yield for are also obtained for aqueous 40% ethanol i.e. TPC (42.54 mg GAE/g DW) and TFC (60.75 mg QE/gDW), respectively. The results suggest that the polarity of the solvents used affect the efficiency of the polyphenol extraction. The polarity index for water, ethanol and isopropanol are 9.0, 4.3 and 3.9, respectively. The result shows that a mixture of low and high polarity solvent produced a higher extraction yield owing to its ability to extract both the hydroxylated and methoxylated compounds. Hydroxylated compound such as phenolic acid is easier to dissolve in water; meanwhile the methoxylated

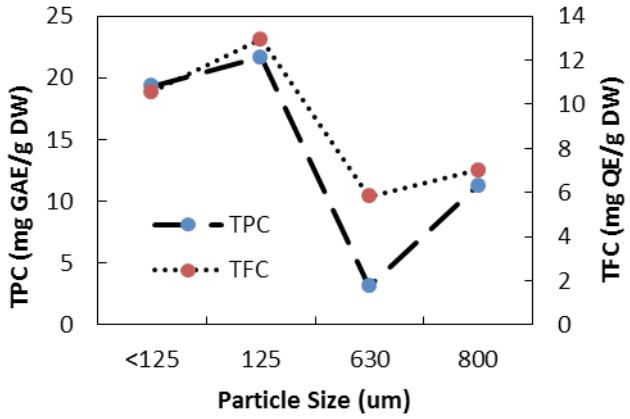

Figure 1. Effect of particle size of TPC and TFC yield from UAE.

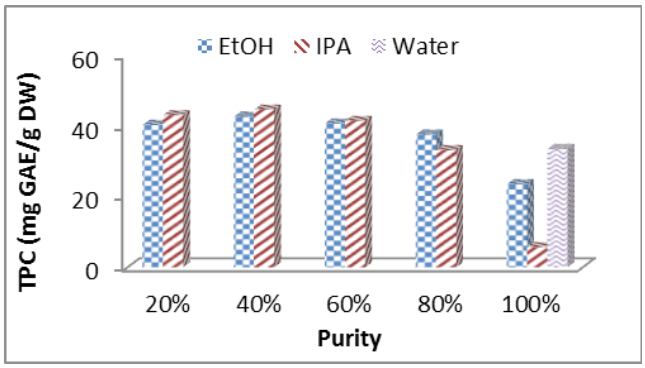

Figure 2. Effect of solvent type and concentration of TPC yield from UAE.

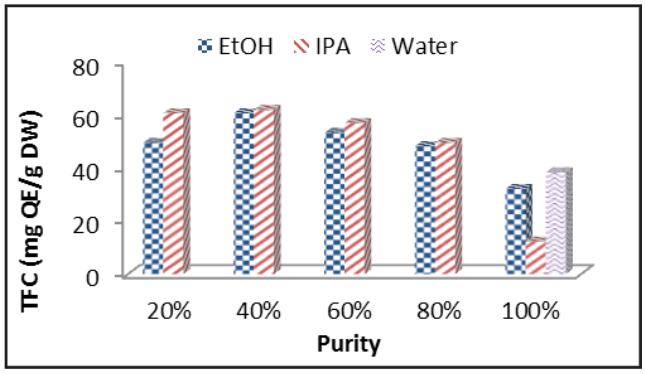

Figure 3. Effect of solvent type and concentration of TFC yield from UAE.

compound such as flavonoid is easier to dissolve in the lower polar solvent. Other researchers[1,2] also presented the same finding on the effect of solvent on TFC and TPC yield from *P. niruri*. Moreover, the major components present in *Phyllanthus* species are active hydrolysable tannins that can be extracted using the ethanol-water mixture because they are semipolar compounds i.e. ellagitannins and gallotannins[13].

3.3 Effect of Ultrasonic Frequency on Phenolic Compound Extraction

The sound waves yielded by the ultrasonic probe that propagates into the liquid media result in an alternating high pressure (compression) and low pressure (rarefaction) cycles. The rate of the cycle depends on the frequency of the sonication wave. Cell envelope of the plant can be mechanically broken by the cavitation shear force generated from the ultrasonic which facilitates the release of the active compound to the solvent. The effect of the frequency changed on the TPC and TFC yield is shown in the Figure 4. The TPC and TFC yield is higher (>50%) at the frequency of 53 kHz than at a frequency of 35 kHz. In the low-pressure cycle, high intensity ultrasonic wave creates the voids or vacuum bubbles in the liquid. Cavitation phenomenon happed when the bubbles attain a volume where they no longer absorb energy, causing them to collapse violently during the high-pressure cycle. Their destructive effect to the plant cell depends on the sonication parameters employed. An increase in the ultrasonic cavitation rate facilitates particle size reduction resulting in a larger surface contact area between the solid and liquid phase, hence enhancing extraction. In[14] also found the optimum frequency band of 56-68 kHz for extraction of

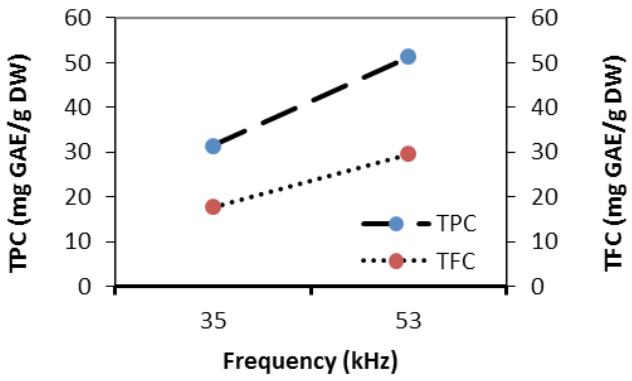

Figure 4. Effect of UAE frequency on TPC and TFC extraction from P. niruri.

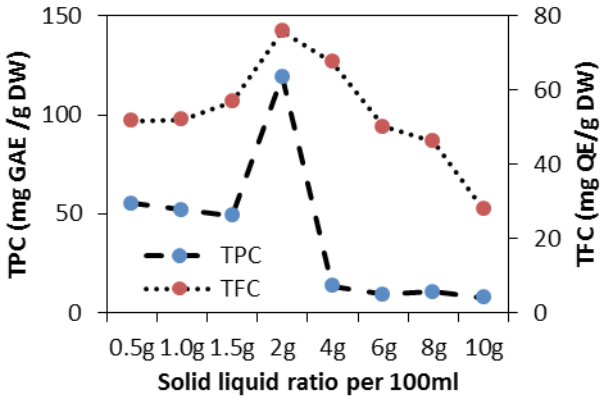

Figure 5. Effect of solid liquid ratio on TPC and TFC extraction from *P. niruri*.

rutin from *Sophara japonica* which is essentially comparable to the findings in this work that 53 kHz has a better yield than that of lower frequency setup.

3.4 Effect of solid liquid ratio on phenolic compound extraction

Apart from the sonication frequency, the solid ratio also affects the extraction yield. Figure 5 shows that the optimum solid liquid ratio is at 2.0 g dry weight per 100 ml of solvent, which yielded the highest phenolic and flavonoid content yield is 119.28 GAE mg/g DW and 75.86 QE mg/g DW, respectively. Figure 5 shows that the solid ratio greatly affects the extraction yield, whereby the polyphenols yield increased up to 60% when the ratio increased from 0.5 g/100 ml to 2 g/100 ml then decline drastically when the ratio increased to 10 g/100 ml. A lower solid to solvent ratio (i.e. 5 g/100 ml) is ineffective because the equilibrium is shifted below the solubility limit of the polyphenol in the solvent. Meanwhile, too much solid to solvent ratio (10 g/100 ml) causing the extraction to be limited by the polyphenol solubility in the solvent and hence not all polyphenol can be extracted from the plant matrix.

4. Conclusion

The highest phenolic and flavonoid content of 119.28 GAE mg/g DW and 75.86 QE mg/g DW were obtained from UAE using a 2 g/100 ml solid ratio. It was found that the particle size of 125 μm has the highest extraction yield compared to other particle size. A mixture of water and a lower polar solvent such as ethanol and isopropanol was

found to give the highest simultaneous extraction of both TPC and TFC. However, the aqueous 40% EtOH should be used for polyphenol extraction from *P. niruri*, because ethanol is a US FDA GRAS solvent. The higher sonication frequency i.e. 53 kHz yielded higher polyphenol yield extraction from *P. niruri*.

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