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Cold Extraction of *Phyllanthus Niruri, Chromolaena Oodorata, Melastoma Malabathricum & Azadirachta Indica* Plants Via High-Pressure Processing (HPP): Evaluation of Physiochemical Properties and Antioxidant Activity

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Abstract: *Phyllanthus niruri*, *Chromolaena odorata*, *Melastoma malabtahricum*, and *Azdirachta indica* are among hundreds of medicinal plants in the world. These plants have been used for many medicinal purposes due to their bioactive compounds. Traditional extraction which involves boiling at high temperature can result in the loss of some bioactive compound including phenolic compound who contribute to the antioxidant activity due to denaturation caused by heat. Therefore, as an alternative, High-Pressure Processing (HPP) was employed as to minimize any quality denaturation during processing. HPP was carried out at two different pressures (200 and 600 MPa) with three different holding times (5, 10, and 15 minutes) respectively. Traditional method at boiling temperature of water (100ºC) for 30 minutes was also carried out as a comparison. The samples were analyzed in terms on its physiochemical properties (pH and color), antioxidant activity, and total phenolic content (TPC). The antioxidant activity and TPC were analyzed using DPPH radical scavenging method and Folin-Ciocalteau reagent respectively. HPP-treated of *A. indica* plant extract exhibits the highest antioxidant activity at 600 MPa/10 min registered 88.55 ± 0.04 % scavenging activities whilst the highest TPC was observed to be HPP-treated of *A. indica* at 200MPa/5min. Based on the results obtained, HPP-treated at 600 MPa with 10 minutes holding time was found to be the ideal or optimum parameters for antioxidant activity. As a conclusion, indeed HPP has the potential to be used as extraction method for plants material.

Keywords: *Phyllanthus Niruri, Chromolaena Odorata, Azadirachta*

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Indica, *Melastoma Malabathricum*, Cold Extraction, High-Pressure Processing, Antioxidant, TPC, Physiochemical Properties

1. Introduction

Herbs, according to Oxford Dictionaries, are any plant with leaves, seeds, or flowers that are primarily used for flavouring, food, medicine, and other purposes. Consumers and researchers are becoming more interested in researching the usage of herbs for therapeutic purposes and daily use thanks to the numerous benefits given. As a result, the Malaysian government included six different types of herbs in its EPP1 (Entry Point Projects: High Value Product) NKEA (National Key Economic Area) development program. NKEA plants include *Phyllanthus niruri, Orthosiphon stamineus,* and *Labisia pumila* [1].

Phyllanthus niruri or popularly known to the Malays as 'dukong anak' is an herbal plant native to a tropical climate like Malaysia. It grows abundantly in Malaysia as it does not have a specific growth requirement. It has been reported that this plant has a broad range of properties such as antiviral activities against hepatitis B, antimicrobial, hepatoprotective, anticancer, and hypocalcemic agent. Besides that, several active phytochemicals for instance flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins also have been discovered in *P. niruri* [2].

Chromolaena odorata is a weed belonging to the Asteraceae family. *C. odorata* is commonly known to the Malays as 'Kapal Terbang' is frequently used as a cure in skin diseases such as skin burns, skin infection and soft tissue wounds in Malaysia. Many studies have reported that several parts of *C. odorata* has a broad range of properties like anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant. Furthermore, several active phytochemical components for instance 2 alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids also have been discovered in *C. odorata* [3].

Azadirachta indica or previously known as Neem is an omnipotent tree that is categorized under mahogany family, Meliaceae. *A. indica* is known to the Malays as 'Semambu' in Malaysia. This plant has been extensively used as traditional medicine since prehistoric times for humankind to treat skin diseases such as leprosy, ulcers, gastro intestinal issues, oral care, urinary tract issues, hair problems, diabetes, high blood pressure, and cholesterol [4].

Melastoma malabathricum or also known as 'Senduduk' among local in Malaysia. This plant has gained herbal status in Malay folklore belief especially in Indian, Chinese and Indonesian folk medicines. Scientist found that different parts of this plant that comprises of the leaves, barks, seeds, roots and shoots have been used in the treatment of a number of common disease/illness including diarrhoea, dysentery, hemorrhoids, cuts and wounds, toothache, and stomachache. Furthermore, this plant also expresses wide pharmacological action such as anti-nociceptive, anti-inflammatory, wound healing, antidiarrheal, cytotoxic, and antioxidant activities.

Recent years have seen the rise of studies on various method for the extraction of plant material such as cold maceration soxhlet method, and traditional extraction. Conventional thermal processing with a higher temperature than 60°C will degrade most of bioactive components in plants. An alternative method is needed to be explored in order to produce a higher quality, a cost-effective, and sustainable method. Therefore, High-pressure Processing (HPP), a novel non-thermal food technology been claimed to have the capacity to recover most of the bioactive compounds in plant material. The application of HPP as a cold-extraction method for plant materials is relatively new but, with promising potential. By studying the effectiveness of HPP on the overall quality of plants extract, particularly on physiochemical and antioxidant activity, it will

help the industrial key players outside to produce a very high quality and sustainable products of food, cosmeceutical, dietary, and other health-related products.

Therefore, this study employed HPP as a technique of cold extraction for different plants namely *P. niruri, A. indica, M. malabathricum* and *C. odorata*. The extracts from HPP treated will be compared to the traditional method in terms of its physicochemical properties (pH and colour), antioxidant activity and also total phenolic content (TPC).

2. Materials and Methods

2.1 Material and Sample Preparation

In this research, *Phyllanthus niruri*, *Chromolaena odorata, Azadirachta indica, and Melastoma malabathricum* leaves were collected from the Nasuha Herbs & Spice Farm in Muar, Johor, Malaysia. *C. odorata* and *A. indica* were washed under running tap water, cut only healthy plants, and brushed away insects. The leaves were rinsed and dried in ovenat temperature of 45°C for 4–7 days or until completely dry [5], then pulverized using a grinder. The respective powders were weighed and stored in an air-tight container until used.

2.2 Extraction (High-Pressure Processing and Traditional)

2.2.1 High Pressure Processing (HPP)

All samples were prepared in 80 ml polyethylene terephthalate (PET) bottles. The bottles next were introduced into a HPP unit (Avure Technologies, Ohio, USA) and distilled water was used as the medium in the chamber. This process was conducted in Universiti Putra Malaysia, in Serdang, Selangor, Malaysia due to unavailability of HPP unit in UTHM. During HPP, the bottles were deposited into the pressure vessel and then subjected to different conditions of processing time (5,10,15 minutes) and pressure (200 and 600 MPa). All extractions were performed at 20ºC (cold extraction). However, adiabatic heating due to pressure increment still happening but the increment is still not enough to promote the thermal degradation of bioactive compounds. After HHP processing, the mixtures were filtered through filter paper (Whatman No. 4) and the filtrates were collected and kept at -20 ºC in cold room until analysis.

2.2.2 Traditional

5 g of powdered *P. niruri, C. odorata, M. malabathricum,* and *A. indica* were mixed with 100 ml of distilled water in a beaker. The beaker containing the mixture was placed on the hot plate and a magnetic stirrer was used to ensure the mixture is continuously and homogenously mixed during the extraction process. After 30 minutes of boiling, the solution mixture was allowed to cool at room temperature. The sample next undergoes the 10 minutes of centrifugation process which involved 10 000 rpm at 26°C

2.3 Determination of Physiochemical Properties

2.3.1 Determination of pH

10 ml of samples from both HPP and traditional method were introduced into a beaker. The pH of the extracts was determined using Apera instruments, pH700 pH meter following standard analytical method. The pH meter was calibrated using buffer solutions of pH 4,7, and 10. The pH of each extract subsequently determined. The results were recorded in duplicate.

2.3.2 Determination of Colour

A Colourimeter (model Colour Quest XE, Hunter Lab, Reston, VA) was used to measure the colour parameters including L* (0 black, 100 white), a* (-a* greenness, +a* redness), and b* (b* blueness, þb* yellowness) for all extract samples. Plants extract samples were placed in a transparent plastic container and measured with the Hunter Lab equipment. L*, a*, b* values were directly obtained from the equipment. Consequently, a*and b* values were used to calculate the browning index (BI) and total colour difference (TCD) based on *Eq.1* and *Eq.2* respectively [6].

$$\mathrm{BI} = \frac{100\big((\times - 0.31)\big)}{0.172} \qquad Eq.1$$

Where

$$\mathrm{x} = (\mathrm{a}* + 1.75L*)/(5.645L* + \mathrm{a}* - 3.012\mathrm{b}*$$

$$\mathrm{TCD} = \sqrt{[(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \qquad Eq.2$$

2.3.3 Determination of Total Phenolic Content (TPC)

Total phenolic content of the plants extract was determined using Folin-Ciocalteu reagent. 100 µL of extracts solution (1 mg/mL) was added, followed by 5 mL of 1:10 Folin-Ciocalteu reagent and mixed for 5 minutes and followed by 4 ml of 75mg/l of sodium carbonate into the solution. The solution has been incubated in water bath at 40ºC for 30 minutes after it has been mixed. After 30 minutes, the absorbance of the mixture was measured at 760 nm against a methanol blank by UV-Vis spectrophotometry. Standard calibration curve being provided by using Gallic acid. Lastly, the TPC of plants extract samples were expressed in mg of Gallic acid equivalents (GAE)/100 ml of sample.

2.3.4 Determination of Antioxidant Activity

The determination of antioxidant activity for this study was by DPPH assaymethod used by [7]. In this method, 1.0 ml of 0.135 mM DPPH was prepared in methanol and mixed with 1.0 ml of plant extracts. The mixture has been vortex-mixed and left to standat 25℃ in the dark for 30 minutes. Absorbance at 517 nm was measured with the useof UV-VIS spectrophotometer with methanol as blank and mixture of distilled water DPPH in methanol as a control. The antioxidant activity value has been determined by using *Eq.3* with AA (%) is antioxidant activity, Abscontrol is the absorbance control reading and Abssample is the absorbance sample reading:

$$\mathrm{AA}\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \qquad Eq.3$$

3. Results and Discussion

3.1 Evaluation on Physiochemical Properties of Different Extraction Methods of *Phyllanthus niruri*, *Chromolaena odorata*, *Azadirachta indica*, and *Melastoma malabathricum*

Two (2) different extraction methods were used in this research, traditional (thermal) method and High Pressure Processing (HPP) method. HPP was conducted at different pressure (200 and 600 MPa) and different holding time (5, 10, 15 minutes) respectively. The effect of these extraction methods on physiochemical properties (pH and colour), antioxidant activity, and total phenolic content were evaluated. The results are presented in the following sections.

3.1.1 Comparison of pH

The results presented in Table 1 shows the comparison of pH for HPP-treated of plants extract of *P. niruri, C. odorata, A. indica,* and *M. malabathricum* in comparison to thermally-treated.

For HPP-treated sample, result shows that *A. indica* at 200MPa/10min has the highest pH value (5.56 ± 0.01) whereas the lowest was observed to be *P. niruri* treated with 200MPa/5min and 600MPa/10min with equal pH value of 3.46 ± 0.01. whilst, for traditional-treated sample, the highest pH was observed to be *A. indica* (4.06 ± 0.01) whereas *P. niruri* was the lowest (2.93 ± 0.01). In general, all plants extract in this study were found to be acidic since the pH ranged from 3-6, less than 7

Table 1: Comparison of pH for HPP and traditional treated plants samples of *Phyllanthus niruri, Chromolaena odorata, Azadirachta indica,* and *Melastoma malabathricum* at different Pressure with different Processing Time

Extraction Method	pH															
	Phyllanthus niruri				*Chromolaena odorata*				*Azadirachta indica*				*Melastoma malabathricum*			
	200 MPa				600 MPa				200 MPa				600 MPa			
	200 MPa				600 MPa											
	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min
HPP	3.46± 0.01	3.47 ± 0.01	3.47± 0.01	3.46 ± 0.01	5.42± 0.01	5.45 ± 0.00	5.44 ± 0.01	5.45 ± 0.01	5.56 ± 0.02	5.56 ± 0.01	5.54 ± 0.00	5.55 ± 0.01	3.75 ± 0.00	3.76 ± 0.00	3.74 ± 0.01	3.75 ± 0.01
Traditiona1	2.93 ± 0.01				4.20 ± 0.01				4.06 ± 0.01				4.10 ± 0.00			

According to previous researches, there is no search finding that shows HPP increases the pH of plant extract in general. The effect of HPP on pH of plant extract is not directly discussed. As a result, the pH of *P. niruri, A. indica, C. odorata,* and *M. malabathricum* HPP-treated plant samples may differ from that of thermally-treated plant samples, although more study is needed to establish this. A study by [8] stated that HPP treatment had no effect on the pH of a lemongrass-lime mixed beverage.

3.1.2 Changes in Colour

Table 2 shows the BI and TCD of HPP-treated in comparison to thermally-treated at different pressure with different processing time.

Table 2: Browning Index (BI) and Total Colour Different (TCD) of HPP-treated of *Phyllanthus niruri, Chromolaena odorata, Azadirachta indica,* and *Melastoma malabathricum* in Comparison to Thermally-treated at different Pressure with different Processing Time

		Colour															
		Phyllanthus niruri				*Chromolaena odorata*				*Azadirachta indica*				*Melastoma malabathricum*			
	Extraction Method	200 MPa		200 MPa 60MPa		600 MPa		200 MPa		600 MPa		200 MPa		600 MPa			
		5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min
BI	HPP	284.88	313.95	284.88	255.81	255.81	348.84	587.21	325.58	197.67	226.74	151.16	174.42	383.72	325.58	447.67	343.02
	Traditional	319.77				151.16				319.77				377.91			
TCD	HPP	0.07	0.09	0.12	0.12	0.01	0.03	0.01	0.03	0.06	0.04	0.12	0.15	0.11	0.05	0.01	0.08
	Traditional	0.05				0.15				0.02				0.10			

In terms of TCD, HPP at 600 MPa with 10 minutes holding time had the most influence on TCD value, while HPP at 200 MPa with 5 minutes holding time had the least impact. Thankfully, TCD values for HPP-treated were within the range that may be characterised as visible, which ranges from 1.5 to 3.0, with a human eye threshold for significant change above 3 [9].

The highest BI for *P. niruri* and *A. indica* was obtained at 200MPa/10min, 200Mpa/15 min for *C. odorata*, and 200MPa/5 min for *M. malabathricum* 200MPa/5min respectively. In general, HPP-treated of *P. niruri and A. inidca* were the only extracts that have lower BI than thermally-treated, which was theoretically logical since lower browning index exhibits higher antioxidant activity. Yuan *et al* in his study suggested that lower browning index may have good antioxidant activity after he and other authors found that peppermint extract treatments reduced phenolic biosynthesis but enhanced antioxidant activity [10].

3.2 Evaluation on Antioxidant Acrivity of Different Extraction Methods of *Phyllanthus niruri*, *Chromolaena odorata*, *Azadirachta indica*, and *Melastoma malabathricum*

Table 3 shows the antioxidant activity of HPP-treated in comparison to thermally-treated. For HPP-treated of P. niruri, and C. odorata, A. indica, and M. malabathricum, all results showed similar pattern where HPP-treated at 600 MPa/10min exhibits the highest antioxidant activity with 47.15±16.33%, 74.98±0.57%, 88.55 ± 0.04%, and 29.64 ± 1.08% respectively.

Table 3: Antioxidant Activity of HPP-Treated of *Phyllanthus niruri, Chromolaena odorata, Azadirachta indica,* and *Melastoma malabathricum* in Comparison to Thermally-Treated at different Pressure with different Processing Time

Extraction Method	Antioxidant Activity (AA) (% Scavenging)															
	Phyllanthus niruri				*Chromolaena odorata*				*Azadirachta indica*				*Melastoma malabathricum*			
	200 MPa		200 MPa 600MPa	600 MPa	200 MPa				600 MPa			200 MPa			600 MPa	
	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min
HPP	43.43 ±2.35	45.65±7.75	46.74 ±15.10	47.15 ±16.33	52.77± 10.71	66.93 ±11.03	68± 6.14	74.98± 0.57	56.32 ±0.07	55.81 ±0.09	87.54 ±0.21	88.55 ±0.04	21.56±6.68	27.79±7.99	28.62±0.06	29.64±1.08
Traditional	8.62±0.04				1.40±0.75				20.12±0.17				26.93±1.26			

All HPP-treated plants extract of *P. niruri, C. odorata, A. indica, and M. malabathricum* exhibited outstanding antioxidant prowess especially for *A. indica* where the HPP-treated at 600 MPa with 10 minutes holding time registered 88.55 ± 0.04 % scavenging activities, being the highest whilst *P. niruri* treated at 200 MPa with 5 minutes holding time was the lowest with 21.56 ± 6.68 % scavenging activities, which still considered to be a high value.

The high antioxidant activity of plants extract especially *C. odorata* and *A. indica,* indicates high content of bioactive compounds. The application of high pressure of 600MPa resulted in highest antioxidant activity. Greater antioxidant activity was reported by [11] who demonstrated that for pressurised strawberry purée at 500 and 600 MPa, the mean anti-radical power values were greater than those of thermally treated samples.

In addition, a number of studies have found that HPP can increase the release of antioxidant chemicals from plant materials owing to cellular structural disturbance. As a result, high-pressure circumstances can cause cell walls to degrade, resulting in the release of intracellular antioxidants such phenolic chemicals and flavonoids into the surrounding solvent. This enhanced antioxidant availability correlates to greater measured antioxidant activity.

Based on the results obtained, it can be concluded that HPP at 600MPa with 10 min holding time was the optimum parameter for antioxidant activity of plants extract of *P. niruri, C. odorata, A. indica,* and *M. malabathricum.*

3.3 Evaluation on Total Phenolic Content (TPC) of Different Extraction Methods of *Phyllanthus niruri, Chromolaena odorata, Azadirachta indica,* and *Melastoma malabathricum*

The total phenolic content (mg GAE/g) of *P. niruri, C. odorata, A. indica,* and *M. malabathricum* HPP-treated in comparison to thermally-treated are tabulated in Table 4 below.
For HPP-treated of *P. niruri,* and *C. odorata,* the highest TPC obtained was at 200 MPa/10min with (161.71 ± 0.00 mg GAE/100 g) and 251.53 ± 0.06 mg GAE/100 g respectively. However, in comparison to thermal, the TPC obtained was observed to be higher than HPP. Whilst, for *C. odorata,* the TPC for thermally-treated on the other hand, was observed to be lower than HPP-treated. Moreover, for *A. indica,* the highest TPC obtained was at 200 MPa with 5 minutes holding time with 279.82 ±0.05 mg GAE/100g. In comparison to thermally-treated, the TPC obtained was observed to be slightly lower than HPP-treated. Lastly, for *M. malabathricum,* the highest TPC obtained was at 200 MPa/15min with 29.31 ± 0.01mg GAE/100g and to compare with thermally-treated, unfortunately, it was higher than HPP-treated.

Table 4: Total Phenolic Content (mg GAE/g) of *P. Niruri, C. Odorata, A. Indica,* And *M. Malabathricum* HPP-Treated in Comparison to Thermally-Treated at different Pressure with different Processing Time

Extraction Method	Total Phenolic Content (TPC) (mg GAE/100g)															
	Phyllanthus niruri				*Chromolaena odorata*				*Azadirachta indica*				*Melastoma malabathricum*			
	200 MPa			600 MPa		200 MPa		600 MPa			200 MPa		600 MPa			
	200 MPa			600MPa												
	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min	5 min	10 min	15 min	10 min
HPP	152.45±0.00	161.71±0.00	128.38±0.00	112.64±0.00	218.19±0.02	251.53±0.06	248.75±0.04	238.56±0.02	279.82±0.05	279.31±0.01	265.42±0.01	215.42±0.00	11.71±0.00	8.94±0.00	29.31±0.01	9.86±0.00
Traditional	286.71±0.16				197.82±0.01				262.64±0.04				262.64±0.05			

Theoretically, the application of HPP was predicted to resulted in higher TPC than thermal method. However, HPP-treated of *P. niruri* and *M. malabathricum* expressed lower TPC than thermally-treated. The observed increase in TPC can be ascribed to increased cell permeability caused by the rupture of cell wall and cell membrane hydrophobic interactions, resulting in mass transfer and the release of matrix-bound phenolic chemicals.

The enhancement of nutritional and sensory characteristics is the primary reason for using non-thermal food processing technology. When opposed to heat treatment, HPP treatment can retain the nutritional and sensory qualities of plant extracts, resulting in greater TPC [12].

4. Conclusion

The study aimed for three distinct objectives, to investigate the potential of High-Pressure Processing (HPP) as a cold extraction method for four different plants herbs (*P. niruri, A. indica, M. malabathricum, and C. odarata).* The investigation also sought to compare how HPP and traditional methods affect the physiochemical properties, Total Phenolic Content, and Antioxidant Activity of these plants. This study also wanted to find out the best timeand pressure for extracting these plants using HPP.

Based on the results obtained in previous chapter, this study found that HPP is a good way to extract the plants because it increased the total phenolic content and antioxidant activity more than traditional method.

This study also found that the optimum parameters for the cold extraction of these plants via HPP varied depending on the plant, with different pressure and time parameters required for each plant. HPP-treated at 600 MPa with 10 minutes holding time was found to be the ideal or optimum parameters for antioxidant activity in which HPP-treated of *A. indica* was found to be registered excellent antioxidant activity with 88.55 ± 0.04 % scavenging activities.

The study gives useful information about how HPP can be used to extract natural products without using heat. The outcomes of this study may aid in the creation of novel and inventive waysto extract natural products, which could greatly affect the food and medicine sectors. Indeed, HPP has potential to be used as extraction method for plants material.

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