

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

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DOI: <https://doi.org/10.30880/jst.2025.17.01.006>

Article Info

Received: 15 March 2025

Accepted: 30 May 2025

Available online: 30 June 2025

Keywords

Phyllanthus niruri, *Chromolaena odorata*, *Azadirachta indica*, *Melastoma malabathricum*, high-pressure processing, antioxidant, physiochemical properties

Abstract

Phyllanthus niruri, *Chromolaena odorata*, *Melastoma malabathricum*, and *Azadirachta indica* are among the many medicinal plants found worldwide. These plants have been widely used for medicinal purposes due to their rich bioactive compounds. Traditional extraction methods, such as boiling at high temperatures, can lead to the degradation of certain bioactive compounds, including phenolic compounds, which contribute to antioxidant activity. This degradation occurs due to heat-induced denaturation. As an alternative, High-Pressure Processing (HPP) was employed to minimize quality deterioration during extraction. HPP was conducted at two different pressures (200 MPa and 600 MPa) with three holding times (5, 10, and 15 minutes). For comparison, a traditional extraction method was also performed by boiling the samples at 100°C for 30 minutes. The extracts were analyzed for their physicochemical properties (pH and color), antioxidant activity, and total phenolic content (TPC). Antioxidant activity was assessed using the DPPH radical scavenging method, while TPC was determined using the Folin-Ciocalteu reagent. Among the tested samples, HPP-treated *A. indica* extract exhibited the highest antioxidant activity at 600 MPa for 10 minutes, achieving 88.55 ± 0.04% scavenging activity. The highest TPC was observed in HPP-treated *A. indica* at 200 MPa for 5 minutes. Based on the results, the optimal HPP parameters for maximizing antioxidant activity were found to be 600 MPa with a 10-minute holding time. In conclusion, HPP demonstrates significant potential as an effective extraction method for medicinal plant materials, offering a promising alternative to traditional heat-based extraction techniques. This study is novel in its comparative evaluation of multiple medicinal plant species under varying HPP conditions, providing new insights into parameter optimization for maximizing antioxidant yield.

1. Introduction

Herbs are plants with leaves, seeds, or flowers primarily used for flavoring, food, medicine, and other purposes. In recent years, both consumers and researchers have shown increasing interest in exploring the therapeutic and everyday applications of herbs due to their numerous benefits. As a result, the Malaysian government has incorporated six different types of herbs into its EPP1 (Entry Point Projects: High-Value Product) under the NKEA (National Key Economic Area) development program. Among these, notable NKEA plants include *Phyllanthus niruri*, *Orthosiphon stamineus*, and *Labisia pumila*.

Phyllanthus niruri, commonly known as dukong anak among Malaysians, is an herbal plant native to tropical climates such as Malaysia. It thrives abundantly in the region as it does not require specific growth conditions. This plant has been reported to possess a wide range of medicinal properties, including antiviral activity against hepatitis B, antimicrobial, hepatoprotective, anticancer, and hypocalcemic effects. Moreover, several active phytochemicals, such as flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins, have been identified in *P. niruri*. [1],[2].

Chromolaena odorata, a weed belonging to the Asteraceae family, is commonly known as Kapal Terbang among Malaysians. It has been traditionally used to treat various skin conditions, including burns, infections, and soft tissue wounds. Numerous studies have reported that different parts of *C. odorata* exhibit a broad spectrum of medicinal properties, such as anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant activities. Additionally, several active phytochemical components, including alkaloids, flavonoids, flavanones, essential oils, phenolics, saponins, tannins, and terpenoids, have been identified in *C. odorata*. [3]

Azadirachta indica, previously known as neem, is an omnipotent tree that belongs to the mahogany family (Meliaceae). In Malaysia, it is commonly referred to as Semambu. This plant has been extensively used in traditional medicine since ancient times for treating various ailments, including skin diseases such as leprosy and ulcers, gastrointestinal issues, oral health problems, urinary tract disorders, hair conditions, diabetes, high blood pressure, and cholesterol management [4].

Melastoma malabathricum, widely known as Senduduk among locals in Malaysia, holds a significant place in Malay folklore and traditional medicine, particularly in Indian, Chinese, and Indonesian herbal practices. Scientists have discovered that different parts of this plant—including the leaves, bark, seeds, roots, and shoots—have been used to treat various common ailments such as diarrhea, dysentery, hemorrhoids, cuts and wounds, toothaches, and stomachaches. Furthermore, this plant exhibits diverse pharmacological activities, including anti-nociceptive, anti-inflammatory, wound-healing, antidiarrheal, cytotoxic, and antioxidant properties [5].

Traditionally, herbal extraction methods, such as decoction (boiling in water) and maceration (soaking in solvents), have been widely used. However, these methods come with several limitations. High-temperature extraction, such as boiling, can lead to the degradation of heat-sensitive bioactive compounds, including phenolics and flavonoids, which are crucial for antioxidant and therapeutic properties. Additionally, prolonged extraction times and the use of organic solvents in maceration may result in the loss of volatile compounds and potential toxicity concerns. Furthermore, traditional methods often yield lower extraction efficiency, require large amounts of solvents, and may not be environmentally sustainable [6].

Given these limitations, this study employed High-Pressure Processing (HPP) as a cold extraction technique for *P. niruri*, *A. indica*, *M. malabathricum*, and *C. odorata*. HPP offers a promising alternative by preserving bioactive compounds while minimizing thermal degradation. This innovative method utilizes high pressure to disrupt cell walls and facilitate the release of valuable phytochemicals without the need for high temperatures, thereby maintaining their potency [7]. Furthermore, HPP has been recognized for its ability to enhance extraction yield and efficiency, reducing extraction times significantly compared to traditional methods [8] (TPC), showcasing its potential as a more effective extraction technique for harnessing the health benefits of herbal products [9]. The extracts obtained from HPP treatment were compared to those produced using traditional extraction methods in terms of their physicochemical properties (pH and color), antioxidant activity, and total phenolic content (TPC) as depicted in Figure 1. This study is the first to check HPP conditions across multiple medicinal plants for enhanced antioxidant yield, offering a better alternative to thermal extraction.

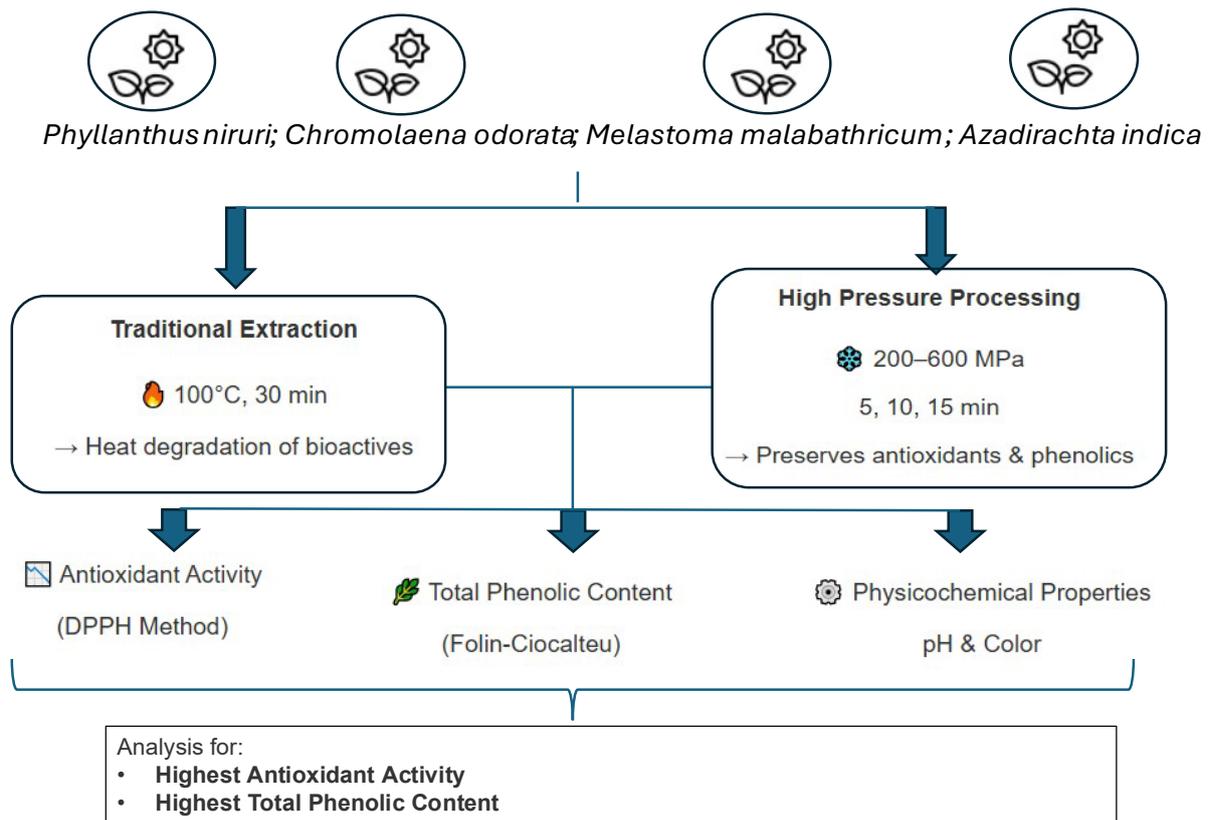


Fig. 1 Graphical abstract for this study

2. Materials and Method

2.1 Material and Sample Preparation

Phyllanthus niruri, *Chromolaena odorata*, *Azadirachta indica*, and *Melastoma malabathricum* leaves were collected from Nasuha Herbs & Spice Farm in Muar, Johor, Malaysia. *C. odorata* and *A. indica* were washed under running tap water. Then, the leaves were rinsed and dried in oven at temperature of 45 °C for 4–7 days or until completely dry [10]. The dried leaves were then pulverized using a grinder. The respective powders were weighed and stored in an air-tight container until used.

2.2 Extraction Process

2.2.1 High Pressure Processing (HPP)

3 g of powdered *P. niruri*, *C. odorata*, *M. malabathricum*, and *A. indica* were mixed with 60 ml of distilled water were prepared in 80 ml polyethylene terephthalate (PET) bottles. The samples subjected to HPP with pressure of 200 MPa at 5, 10, and 15 minutes and 600 MPa at 10 minutes at ambient temperature. The operating conditions were selected based on findings from previous research, which identified this pressure and duration as optimal for extraction using HPP [8],[9]. The temperature within the pressure chamber was monitored using thermocouples that were immersed in the pressure medium (distilled water) where it positioned at the middle and top of the vessel. On the cycle report, the compression time, decompression time, and average temperature were collected directly from a control system handled by a computer running software [11]. The resulted extracts then were cooled in ice water after HPP treatment until used. The experiment used triplicate *C. odorata* and *A. indica* samples from the same batch. After HPP processing, the mixtures were filtered through filter paper (Whatman No. 4) and the filtrates were collected and kept at -20 °C in cold room until analysis.

2.2.2 Conventional Method (Thermal)

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 (where the temperature was monitored at around 98-100 °C) and a magnetic stirrer was used to ensure the mixture is continuously and homogeneously mixed during the extraction process. After 30 minutes of boiling, the solution mixture was allowed to cool at room temperature (approximately 37 °C). The sample next undergoes the 10 minutes of centrifugation process (10 000 rpm at 26 °C) to separate the solid residues (pellet) from the liquid extract (supernatant). By removing suspended particles, debris, and undissolved plant materials, ensuring a clear solution for further analysis [12].

2.3 Determination of Physicochemical 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 pH meter (Apera instruments, pH700) 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 samples were tested thrice, and the results were recorded.

2.3.2 Determination of Colour

A colorimeter (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, βb^* 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 [11].

$$BI = \frac{100((x - 0.31))}{0.172} \quad Eq. 1$$

Where

$$x = (a^* + 1.75L^*) / (5.645L^* + a^* - 3.012b^*)$$

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

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 75 mg/l of sodium carbonate into the solution. The solution has been incubated in water bath at 40 °C 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 [12].

2.3.4 Determination of Antioxidant Activity

The determination of antioxidant activity for this study was by DPPH assay method used by [12]. 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 stand at 25 °C in the dark for 30 minutes. Absorbance at 517 nm was measured with the use of 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, $Ab_{S_{control}}$ is the absorbance control reading and $Ab_{S_{sample}}$ is the absorbance sample reading:

$$AA (\%) = \frac{Ab_{S_{control}} - Ab_{S_{sample}}}{Ab_{S_{control}}} \times 100 \quad Eq. 3$$

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 pH for HPP-treated and Thermally Treated of Plants Extract

The results presented in Table 1 show 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, the 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 extracted in this study were found to be acidic since the pH range was from pH3 to pH6.

Table 1 Comparison of pH for HPP and conventional treated samples of *Phyllanthus niruri*, *Chromolaena odorata*, *Azadirachta indica*, and *Melastoma malabathricum* The \pm values represent the standard deviation calculated from the average readings of replicated samples

Treatment/ samples	<i>P. niruri</i>	<i>C.odorata</i>	<i>A. indica</i>	<i>M. malabathricum</i>
HPP				
200MPa, 5 min	3.46 ± 0.01	5.42 ± 0.01	5.56 ± 0.01	3.75 ± 0.00
200MPa, 10 min	3.47 ± 0.01	5.45 ± 0.01	5.56 ± 0.01	3.76 ± 0.00
200MPa, 15 min	3.47 ± 0.01	5.44 ± 0.01	5.54 ± 0.01	3.74 ± 0.01
600MPa, 10 min	3.46 ± 0.01	5.45 ± 0.01	5.55 ± 0.01	3.75 ± 0.01
Conventional (Thermal)	2.93 ± 0.01	4.20 ± 0.01	4.06 ± 0.01	4.10 ± 0.00

Previous research indicates that there is currently no evidence demonstrating that high pressure processing (HPP) consistently increases the pH of plant extracts. While the impact of HPP on the pH of plant extracts has not been directly addressed, it is important to recognize that various factors, such as the type of plant material, extraction methods, and storage conditions, can significantly influence pH levels. Consequently, the pH of HPP-treated samples of *P. niruri*, *A. indica*, *C. odorata*, and *M. malabathricum* may differ from that of thermally treated samples, though further studies are necessary to confirm this. Besides, the variation in pH observed among the plant extracts following HPP and thermal treatments can be attributed, in part, to differences in the structural composition of the plant materials. For instance, *A. indica*, which consistently exhibited higher pH values across both treatments, may possess a cellular structure or biochemical profile that resists acidification which is possibly due to stronger buffering capacity or lower concentrations of organic acids. In contrast, *P. niruri*, which showed the lowest pH values, might have a higher content of organic acids or a more permeable cell structure that allows greater release of acidic compounds during processing. The impact of pressure and time on cellular disruption can also differ depending on the toughness of the plant matrix, which affects how intracellular contents are released during HPP. These structural and compositional differences are key in explaining the pH outcomes observed in the study [9]. However, a study cited in reference [13] reported that HPP treatment did not affect the pH of a lemongrass-lime mixed beverage.

3.1.2 Colour for HPP-treated and Thermally Treated of Plants Extract

Table 2 presents a comparison of the browning index (BI) and total color difference (TCD) of HPP-treated samples versus conventionally-treated samples, analyzed at varying pressures and processing times. Regarding TCD, HPP treatment at 600 MPa with a holding time of 10 minutes exhibited the most significant effect on TCD values, while HPP at 200 MPa for 5 minutes had the least impact. Notably, the TCD values for HPP-treated samples fell within the range considered visible, which is between 1.5 and 3.0, with a human perceptual threshold for significant change set above 3 [13],[14]. The highest BI values were recorded for *P. niruri* and *A. indica* at 200 MPa for 10 minutes, for *C. odorata* at 200 MPa for 15 minutes, and for *M. malabathricum* at 200 MPa for 5 minutes. Overall, HPP-treated extracts of *P. niruri* and *A. indica* displayed lower BI values compared to their conventionally-treated counterparts, which align with the theoretical understanding that a lower browning index is associated with higher antioxidant activity. Yuan et al. suggested that a reduced browning index may indicate enhanced antioxidant activity, as their research indicated that peppermint extract treatments decreased phenolic biosynthesis while increasing antioxidant activity [14], [15]. For instance, *P. niruri* and *A. indica* exhibited lower BI values after HPP treatment compared to conventional heating, which may be due to better preservation of their cellular structure under high pressure. This structural integrity helps limit the release and oxidation of phenolic compounds, which are precursors to enzymatic browning. Additionally, HPP tends to inactivate browning-related enzymes more effectively without causing significant thermal degradation of pigments, unlike conventional heat treatments. Conversely, higher BI values at certain HPP conditions may result from prolonged exposure leading to cell wall disruption and release of phenolic compounds susceptible to oxidation. The relatively high TCD values observed at 600 MPa/10 min indicate that increased pressure and time may induce more noticeable color changes, possibly due to pigment breakdown or matrix alteration. Still, since these TCD values fall within or just above the perceptibility threshold, the visual impact remains moderate. In summary, HPP-treated extracts of *P. niruri* and *A. indica* showed lower BI than thermally treated ones, which is beneficial as it indicates higher antioxidant activity. Studies have suggested that a lower browning index may reflect better antioxidant properties. Overall, HPP may help preserve the beneficial compounds in plant extracts compared to heat treatment, resulting in less browning.

Table 2 (a) Browning Index (BI) and (b) Total Colour Different (TCD) of HPP-treated of *Phyllanthus niruri*, *Chromolaena odorata*, *Azadirachta indica*, and *Melastoma malabathricum* in Comparison to Conventional-treated. The ± values represent the standard deviation calculated from the average readings of replicated samples

		(a) Browning index			
Treatment/ samples		<i>P. niruri</i>	<i>C.odorata</i>	<i>A. indica</i>	<i>M. malabathricum</i>
HPP	200MPa, 5 min	284.88 ±0.01	255.81±0.01	197.6±0.01	383.72±0.00
	200MPa, 10 min	313.95±0.01	348.84±0.01	226.74±0.01	325.58±0.00
	200MPa, 15 min	284.88±0.01	587±0.01	151.16±0.01	447.67±0.01
	600MPa, 10 min	255.81±0.01	325.5 ±0.01	174.42±0.01	343.02±0.01
Conventional (Thermal)		319.77 ± 0.01	151.16 ± 0.01	319.77 ± 0.01	377.91 ± 0.00

(b) Total Color Difference

Treatment/ samples		<i>P. niruri</i>	<i>C. odorata</i>	<i>A. indica</i>	<i>M. malabathricum</i>
HPP	200MPa, 5 min	0.07 ±0.01	0.01±0.01	0.06±0.01	0.11±0.00
	200MPa, 10 min	0.09±0.01	0.03±0.01	0.04±0.01	0.05±0.00
	200MPa, 15 min	0.12±0.01	0.01±0.01	0.12±0.01	0.01±0.01
	600MPa, 10 min	0.12±0.01	0.03 ±0.01	0.15±0.01	0.08±0.01
Conventional (Thermal)		0.05 ± 0.01	0.15 ± 0.01	0.02 ± 0.01	0.10 ± 0.00

3.2 Antioxidant Activity for HPP-treated and Thermally Treated of Plants Extract

Table 3 presents a comparison of antioxidant activity between high-pressure processing (HPP) and thermal treatments for various plant extracts, specifically *P. niruri*, *C. odorata*, *A. indica*, and *M. malabathricum*. The results indicate a consistent trend across the HPP-treated samples, with the highest antioxidant activity observed at 600 MPa for 10 minutes, yielding values of 47.15±16.33 %, 74.98±0.57 %, 88.55±0.04 %, and 29.64±1.08 % respectively. These findings might be attributed to pressure-induced cell wall disruption. The mechanical breakdown of cellular structures enhances the release of intracellular antioxidants into the solvent, increasing their measurable activity. Notably, *A. indica* demonstrated exceptional antioxidant capacity, achieving an impressive 88.55±0.04 % scavenging activity under these conditions, suggests that their tissue structure is particularly responsive to pressure, allowing for efficient extraction of bioactive compounds. This plant likely possesses a rich matrix of polyphenolic compounds that are more readily released when their cell walls and membranes are compromised by high pressure. In contrast, *P. niruri* treated at 200 MPa for 5 minutes exhibited the lowest scavenging activity at 21.56±6.68%, which, nonetheless, is still considered a significant value. This might be due to a more rigid or less responsive cellular structure that restricts compound release under milder HPP conditions. However, even in this case, increasing the pressure to 600 MPa improves compound availability, supporting the idea that structural breakdown under pressure is a key factor in enhancing antioxidant recovery.

Table 3 Antioxidant Activity (% Scavenging) of HPP-Treated of *Phyllanthus niruri*, *Chromolaena odorata*, *Azadirachta indica*, and *Melastoma malabathricum* in Comparison to Thermally Treated. The ± values represent the standard deviation calculated from the average readings of replicated samples

Treatment/ samples		<i>P. niruri</i>	<i>C. odorata</i>	<i>A. indica</i>	<i>M. malabathricum</i>
HPP	200MPa, 5 min	43.43±2.35	52.77±10.71	56.32±0.07	21.56±6.68
	200MPa, 10 min	45.65±7.755	66.93±11.03	55.81±0.09	27.79±7.79
	200MPa, 15 min	46.74±5.10	68±6.14	87.54±0.21	28.62±0.06
	600MPa, 10 min	47.15±6.63	74.98±0.57	88.55±0.04	29.64±1.08
Conventional (Thermal)		8.62 ± 0.04	1.40 ± 0.75	20.12± 0.17	26.93 ± 1.26

The high antioxidant activity observed in the extracts, particularly in *C. odorata* and *A. indica*, suggests a rich content of bioactive compounds. The application of 600 MPa of pressure maximized the antioxidant activity, corroborating findings from previous studies, such as [16], which reported that pressurized strawberry purée at 500 and 600 MPa exhibited higher mean anti-radical power values compared to thermally treated samples. Furthermore, multiple studies have indicated that HPP can enhance the release of antioxidant compounds from plant materials as a result of cellular structural disruption. High-pressure conditions can lead to the degradation

of cell walls, facilitating the release of intracellular antioxidants like phenolic compounds and flavonoids into the surrounding solvent. This increased availability of antioxidants contributes to the observed enhancement in antioxidant activity. The antioxidant activity observed in the various plant extracts following HPP and thermal treatments can be closely associated with the structural characteristics and phytochemical composition of the plant materials. It is important to note that the structural integrity of plant tissues plays a crucial role in determining the availability and extractability of antioxidant compounds such as phenolics, flavonoids, and other secondary metabolites. Thermal treatments, in contrast, may not facilitate the same level of structural disruption without risking degradation of heat-sensitive antioxidants. Overall, the differences in antioxidant activity reflect not only the inherent phytochemical content of each plant but also how their unique structures respond to processing conditions. In conclusion, the results affirm that HPP at 600 MPa with a holding time of 10 minutes is the optimal parameter for maximizing antioxidant activity in the plant extracts of *P. niruri*, *C. odorata*, *A. indica*, and *M. malabathricum*.

3.3 Total Phenolic Content (TPC) for HPP-treated and Thermally Treated of Plants Extract

The total phenolic content (TPC, mg GAE/g) of *P. niruri*, *C. odorata*, *A. indica*, and *M. malabathricum* treated with high-pressure processing (HPP) is compared to those treated with thermal methods, as shown in Table 4 below. For the HPP-treated *P. niruri* and *C. odorata*, the highest TPC recorded was at 200 MPa for 10 minutes, yielding 161.71 ± 0.00 mg GAE/100 g and 251.53 ± 0.06 mg GAE/100 g, respectively. However, the TPC obtained from conventional treatment was greater than that from HPP for both species. In contrast, the TPC of *C. odorata* exhibited lower values for conventional treatment than for HPP treatment. For *A. indica*, the peak TPC was achieved at 200 MPa with a 5-minute holding time, resulting in 279.82 ± 0.05 mg GAE/100 g, which was slightly lower than the HPP-treated samples when compared to their conventional counterparts. Lastly, *M. malabathricum* showed the highest TPC at 200 MPa for 15 minutes, reaching 29.31 ± 0.01 mg GAE/100 g, but this was lower than the TPC for conventionally treated samples. Theoretical predictions suggested that HPP would yield higher TPC results than thermal methods; however, both *P. niruri* and *M. malabathricum* demonstrated lower TPC levels with HPP. The increase in TPC observed in some cases can be attributed to enhanced cell permeability due to the rupture of cell walls and membranes, leading to improved mass transfer and the release of bound phenolic compounds. The primary motivation for employing non-thermal food processing technologies, like HPP, is to enhance nutritional and sensory characteristics, as they are more effective than heat treatment in preserving these qualities in plant extracts, ultimately resulting in higher TPC levels [17].

Table 4 Total Phenolic Content (mg GAE/100g) of *P. niruri*, *C. odorata*, *A. indica*, And *M. malabathricum* HPP-Treated in Comparison to Thermally-Treated. The \pm values represent the standard deviation calculated from the average readings of replicated samples

Treatment/ samples		<i>P. niruri</i>	<i>C.odorata</i>	<i>A. indica</i>	<i>M. malabathricum</i>
HPP	200MPa, 5 min	152.45±0.00	218.19±0.02	279.82±0.05	11.71±0.00
	200MPa, 10 min	161.71±0.00	251.53±0.06	279.31±0.01	8.94±0.00
	200MPa, 15 min	128.38±0.00	248.75±0.04	265.42±0.01	29.31±0.01
	600MPa, 10 min	112.64±0.00	238.56±0.02	215.42±0.00	29.64±1.08
	Conventional (Thermal)	286.71 ± 0.16	197.82 ± 0.01	262.64± 0.04	262.64 ±0.05

In conclusion, while high-pressure processing (HPP) demonstrated potential for enhancing the total phenolic content (TPC) of certain plant extracts, the overall results indicate that thermal treatment often yielded higher TPC levels for most species examined, specifically *P. niruri*, *A. indica*, and *M. malabathricum*. Notably, *C. odorata* showed a better TPC with HPP than with thermal treatment. Although theoretical predictions favored HPP for higher TPC, the findings suggest that the effectiveness of either processing method may vary by species. The observed increases in TPC with HPP in some cases are likely due to improved mass transfer resulting from cell

wall disruption; however, the advantages of HPP in preserving nutritional and sensory qualities must be weighed against its performance compared to thermal methods.

4. Conclusion

This study explored High-Pressure Processing (HPP) as a cold extraction method for *P. niruri*, *A. indica*, *M. malabathricum*, and *C. odorata*, comparing its effects with traditional methods on physicochemical properties, total phenolic content, and antioxidant activity. Findings showed that HPP enhanced total phenolic content and antioxidant activity more effectively than conventional extraction (thermal). The optimal extraction parameters varied by plant, with 600 MPa for 10 minutes yielding the highest antioxidant activity, particularly in *A. indica* ($88.55 \pm 0.04\%$ scavenging activity). This study highlights HPP's potential as an efficient, heat-free extraction method for natural products, offering valuable insights for applications in the food and pharmaceutical industries.

Acknowledgement

The authors would like to thank Universiti Tun Hussein Onn Malaysia (UTHM) for the grant Innovation and Commercialization Incubator – I030. The supply of plant samples from Nasuha Herba & Rempah Karya Sari Sdn Bhd, Johor is much appreciated. Special thanks to staff from the Department of Chemical Engineering Technology, Faculty of Engineering Technology for their technical support.

Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: **study conception and design:** Noor Akhmazillah Mohd Fauzi, Hariz Haikal Nasuha; **data collection:** Nurul Fatihah Ibrahim, Faiz Razali; **analysis and interpretation of results:** Noor Akhmazillah Mohd Fauzi, Nurul Fatihah Ibrahim, Sity Aishah Mansur; **draft manuscript preparation:** Angzzas Sari Mohd Kassim, Aliff Hisyam A Razak, Nurul Fatihah Ibrahim. All authors reviewed the results and approved the final version of the manuscript.

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