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The Effect of Various Sterilization Methods and Volume Containers on Phytochemical Content of Methanol Extract of *Phyllanthus urinaria*

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Abstract

Phyllanthus urinaria is an annual perennial herbal species found in tropical Asia, America, China, and the Indian Ocean islands. *Phyllanthus urinaria* is used in folk medicine as a cure to treat jaundice, diabetes, malaria, and liver diseases. Sterilizing the substrate is a crucial step in the fermentation process. This process ensures that the inoculated microorganism is entirely single. Autoclave sterilization is widely favored within the scientific community. In autoclaving, pressurized steam is employed to deliver heat, effectively reducing the bioactive compounds present in the substrate. Comparative studies on various sterilization methods have been reported. This study aims to investigate the effects of substrate containers in sterilization methods of the herbal plant on phenol and flavonoid compounds by LC-MS (Liquid Chromatography-Mass Spectrometry) analysis. Three sterilization methods (pasteurization, steam, and autoclave sterilization) were each applied to the meniran herbal plant (*Phyllanthus urinaria*) for 15 minutes. Using the aluminum chloride colorimetric assay, the sterilization results were measured for total phenol content, the Folin-Ciocalteu test, and total flavonoid content. The LC-MS analysis showed that the methanol extract of *Phyllanthus urinaria* (APU) sterilized by autoclaving resulted in the most significant reduction in active phenolic and flavonoid compounds. Pasteurization, steaming, and autoclaving in a big container resulted in total flavonoid content of 1.80±0.034, 1.70±0.021, and 1.71±0.029 mg QE/g extract. The total phenolic content was 26.49±0.591, 22.77±0.230, and 22.097±0.155 mg GAE extract/g, respectively. Meanwhile, using a small container, each method produced a total flavonoid content of 1.73±0.024, 1.71±0.051, and 1.62±0.015 mg QE/g extract, respectively. The total phenolic content was 20.56±0.093, 19.79±0.295, and 20.09±0.124 mg GAE/g extract. Furthermore, the LC-MS profile revealed that APU experienced a reduction in ρ-hydroxybenzaldehyde and naringenin compounds, leading to a decrease in rutin, methyl brevifolincarboxylate, and ethyl gallate compounds. From the results of LC-MS analysis, this research determined that pasteurization using a big container is the most effective sterilization method for preserving the highest levels of total flavonoid and phenolic content in *Phyllanthus urinaria* while minimizing adverse effects on phytochemical compounds.

1. Introduction

Phyllanthus urinaria is an annual perennial herbal species found in tropical Asia, America, China, and the Indian Ocean islands. *Phyllanthus urinaria* is used in folk medicine as a cure to treat jaundice, diabetes, malaria, and liver diseases [1]. Previous phytochemical investigations on *Phyllanthus urinaria* have isolated lignans, flavonoids, tannins, and other benzenoid

constituents. The main chemical constituents of this plant are lignin, tannin, and flavonoids [2].

Sterilization of herbal ingredients must be carried out to ensure the safety of herbal medicines (to prevent contamination due to micro bacteria, which may arise during storage, extraction, and subsequent procedures, including fermentation. Many previous studies of sterilization methods have been reported. The autoclave is the most popular sterilization method in the science field. This machine uses steam under pressure to kill harmful bacteria, viruses, fungi, and spores on items placed inside a pressure vessel. However, the autoclave method that uses highly pressurized steam was suspected to reduce the substrate bioactive compounds. The autoclave is still absolutely effective for the sterilization process. Harjanti *et al.* [3] reported that the autoclaved herbal formula (*P. betle* leaves extract (PBE), *C. domestica* extract (CDE), and *C. xanthorrhiza* extract (CZE)) resulted in the reduction of flavonoid and phenolic content was far less compared to with syringe filter.

Nevertheless, Pedrosa *et al.* [4] reported that autoclaving significantly decreased legumes' phenolic compounds. This phenomenon was also reported by Gupta and Chaturvedi [5], who found that the blanched extract of *Helianthus tuberosus* had significantly higher total phenolic and flavonoid contents than autoclaved extract. Research on the effect of container volume with various sterilization methods on bioactive compounds has not been reported.

This study aims to review the effect of various sterilization methods and volume containers on the phytochemical contents of the Meniran herbal plant (*Phyllanthus urinaria*). This research is expected to provide information on which method of sterilization with the right size container can effectively eliminate microbes without damaging the bioactive compound before fermentation.

2. Experimental

2.1. Materials and Tools

Materials used were meniran herbal plant (*Phyllanthus urinaria*) collected from Semarang regency, methanol, distilled water, formic acid, quercetin, $AlCl_3.6H_2O$, CH_3COONa, gallic acid, Folin-Ciocalteu's reagent, and Na_2CO_3.

The equipment used in the sterilization process was an autoclave for autoclaving, a steamer set for steaming and a boiling pan for pasteurization, a stove, substrate containers (330 mL and 50 mL), analytical balance (Ohaus Pioneer PA214), beaker glass (Herma), UV-Vis spectrophotometer, incubator, water bath shaker, erlenmeyer, autoclave, a steamer set, glass vials, Buchner funnel, filtering flask, filter paper, cuvet, and micropipette.

2.2. Sample Preparation

Meniran herbal plant (*Phyllanthus urinaria*) was collected from Semarang Regency, Central Java Province, Indonesia. Plant materials were plant determination at the Biology Department, Diponegoro University. Plant materials were dried and finely ground into a powder [3]. Three grams of the sample were placed into a container. The substrate container comprised the small container and the big container. Then, each was added with 15 mL of distilled water.

2.3. Sterilization

Three sterilization methods were carried out on all samples in big and small containers: pasteurization (70°C), steaming (100°C), and autoclaving (121°C) for 15 minutes. After sterilization, each sample was extracted with methanol and filtered. The filtrates of each sample were stored in a refrigerator before undergoing a total phenol test and total flavonoid test.

2.4. Extraction

The extraction was conducted using the maceration method with 96% methanol (v/v) described by Feitosa *et al.* [6] with some modifications. The container containing 3 g of *Phyllanthus urinaria*, 15 mL distilled water, and 15 mL methanol was stirred in an orbital shaker at 25°C and 125 rpm for 1 hour. Samples were filtered through filter paper and a Buchner vacuum.

2.5. LC-MS Analysis

The Liquid Chromatography-Mass Spectroscopy (LC-MS) analysis was based on the method by Kumar *et al.* [7] with some modifications. The samples, including non-sterilized *Phyllanthus urinaria* (control) and autoclaved extract, were analyzed using LC-MS. The choice of autoclaving for sterilization aligns with standard research practices, particularly in biochemistry. The samples were filtered using 0.22 µm filters before injection. The LC-MS analysis of flavonoid and phenolic was carried out on an Advion C18 column (2.1 mm × 50 mm, 1.8 µm) maintained at 20°C with a flow rate of 0.3 mL/minute. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol). The gradient solvent system was eluted with 90% A, 100% B (0-10 minutes), 100% B (11-16 minutes), and 90% A (16-20 minutes) for equilibration before the next injection. Mass spectrometer conditions included ionization mode as electrospray ionization (ESI) (+), a mass scan range of m/z 100-1500, a capillary temperature of 250°C, source gas temperature of 200°C, and a capillary voltage of 180 V.

2.6. Determination of Maximum Wavelength (λ_{max}) of Quercetin

The absorbance of quercetin solution (60 ppm) was measured at the wavelength range of 400-450 nm. The maximum wavelength was recorded to measure the absorption of the methanol extract of the meniran sample.

2.7. Total Flavonoid Content (TFC)

The determination of the total flavonoid content followed the methodology conducted by Ibrahim *et al.* [8], with some modifications. Initially, a solution of 10% $AlCl_3.6H_2O$ (w/v) and 1 M CH_3COONa in distilled water was prepared. Subsequently, a test sample of 10 mL was created by combining 1 mL of the extract, 3 mL of

methanol, 200 µL of $AlCl_3.6H_2O$, and 200 µL of CH_3COONa, with distilled water added to reach the mark. The resulting samples were incubated for 30 minutes at 25°C. Methanol served as the blank, and quercetin functioned as the standard. The blank comprised all reagents and solvents without the sample solution. Total flavonoid content was determined using a standard quercetin calibration curve. The results were expressed in mg of quercetin equivalent (QE)/g of extract.

2.8. Determination of Maximum Wavelength (λ_{max}) of Gallic Acid

Determination of the maximum wavelength of gallic acid was carried out by measuring the acid solution concentration error of 60 ppm at a wavelength of 400-800 nm using a UV-Vis spectrophotometer.

2.9. Total Phenolic Content (TPC)

The total phenolic content followed the method by Ibrahim *et al.* [8] with modifications. Initially, 10% Folin-Ciocalteu's reagent in distilled water (v/v) and 7.5% Na_2CO_3 in distilled water (w/v) were prepared. Afterward, 0.5 mL of sample was added with 2.5 mL of distilled water and 2.5 mL of Folin-Ciocalteu's reagent. The mixture was incubated at 25°C for 15 minutes and added with 2 mL Na_2CO_3 for 30 minutes. Methanol was used as blank control, and gallic acid was used as standard. The blank consisted of all reagents and solvents without sample solution. The standard gallic acid calibration curve was used to determine the content. The results were given as mg gallic acid equivalents (GAE)/g of extract.

2.10. Statistical Analysis

In this study, a completely randomized design with two replicates was used to compare the total flavonoid and phenolic and the different sterilization methods. Furthermore, a completely randomized design with three replicates was used to compare the total flavonoid and phenolic of three formulas and the different sterilization methods. In addition, a means comparison was performed using the ANOVA single-factor test ($p<0.05$).

3. Results and Discussion

Meniran herbal plant (*Phyllanthus urinaria*) was collected from Semarang Regency, Central Java, Indonesia. Meniran has been tested for plant determination at the Ecology and Biosystems Laboratory, Biology Department, UNDIP, with determination key of 1b-2b-3b-4b-6b-7b-9b-10b-11b-12b-13b-14a-15b-(Gol.9 Dispersed compound leaves)- 197a-198b-200b-201b-202b-203a-Fam Euphorbiaceae -1a-Genus Phyllanthus-1b-3b*Phylanthus urinaria*.

The plant material undergoes an air-drying process, carefully shielded from sunlight, followed by meticulous grinding into a powder and subsequent sieving through a 40-mesh sieve [3]. The purposes of wind-drying and avoiding direct sunlight are to prevent damage from ultraviolet light, known for its potential harm to samples, and to ensure an even distribution of heat throughout the drying process. This drying procedure aims to reduce the water content in the sample, thus preventing bacterial spoilage [9]. Additionally, this step is crucial for preserving the bioactive components of the plant. It is recommended to employ convective drying at relatively low temperatures [10]. After drying, the meniran was ground and sieved to 40 mesh to obtain a fine powder with small particles. This particle size is deliberately pursued to facilitate solvent interaction with active compounds. Smaller particle sizes enhance accessibility for the solvent due to the increased surface area of the powder [11].

Sterilization of herbal ingredients is imperative to guarantee the safety of herbal medicines by preventing contamination from microorganisms, which may occur during various stages such as storage, extraction, and subsequent processes, including fermentation. It is crucial to emphasize that an effective sterilization method should proficiently eliminate microbes without destroying bioactive compounds. Furthermore, the chosen method should be economically viable to facilitate its application in experimental settings and the pharmaceutical industry [3].

The extraction process aims to isolate the chemical compounds present in the sample. It operates on the principle of mass transfer, wherein dissolved substance components move from the sample into the solvent, diffusing at the interfacial layer. In this study, 96% methanol as a polar solvent was employed. The chosen extraction method was maceration due to its simplicity and avoidance of heating, which is known to decrease flavonoid levels. The extract was macerated in an orbital shaker for 15 mL of 96% methanol for an hour. Then, the extract was filtrated using a Buchner vacuum, followed by the determination of total flavonoid and phenolic content.

3.1. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using the Aluminum chloride ($AlCl_3$) colorimetric method. Determination of TFC using $AlCl_3$ is only possible if the metal chelate absorption of individual flavonoids in the sample quantitatively has the same absorption at a certain wavelength [12]. The principle of this method is where Al(III) is utilized as a complexing agent. $AlCl_3$ forms acid-stable complexes with the C-4 keto groups and the C-3 or C-5 hydroxyl group of flavones and flavonols [13].

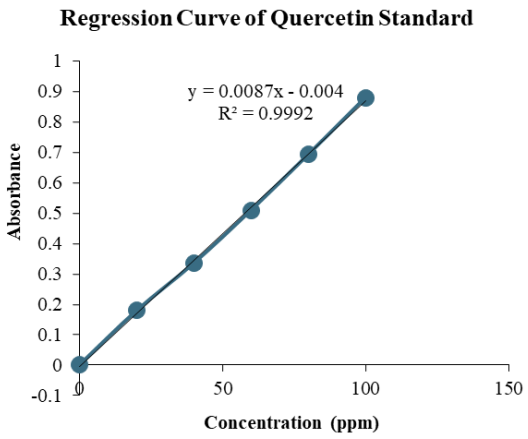

Figure 1. Regression curve of quercetin standard

The total flavonoid content in the meniran methanol extract was determined using a UV-Vis spectrophotometer. This method relies on flavonoids possessing a conjugated aromatic system, causing them to exhibit strong absorption bands within the ultraviolet and visible light spectrum. In this investigation, a standard quercetin solution, with concentrations ranging from 20 to 100 ppm, was employed to ascertain the total flavonoid content in the samples. The spectrophotometer scans were conducted within the 400-450 nm wavelength, resulting in a maximum wavelength of 431 nm. The curve regression is y = 0.087x – 0.004 with R^2 = 0.9992, as shown in Figure 1.

Table 1 presents the effects of different sterilization methods on the concentrations of flavonoids in the *Phyllanthus urinaria*. All sterilization methods led to a reduction in flavonoid content in *Phyllanthus urinaria* (Table 1). Nonetheless, pasteurization in big containers resulted in a considerably smaller decrease. The total flavonoid content in the extract sterilized through pasteurization (PPU) was notably higher ($p<0.05$) when compared to other sterilization methods. Specifically, the pasteurization process using big containers managed to retain approximately 85.41% of the original flavonoid content, as illustrated in Figure 2.

Table 1. The average total flavonoid content (mgQE/g extract) of *Phyllanthus urinaria* after sterilization with different methods

Container's volume	Sterilization method			
	PU (control)	PPU	SPU	APU
Big	2.11±0.03	1.80±0.034*	1.70±0.021*	1.71±0.029*
Small	2.11±0.018	1.73±0.024*	1.71±0.051*	1.62±0.015*

Values are mean ± SD of duplo tests; (*) means significantly different ($p<0.05$);
PU: Control/extract without sterilization
PPU: Pasteurization-sterilized extract
SPU: Steaming-sterilized extract
APU: Autoclave-sterilized extract

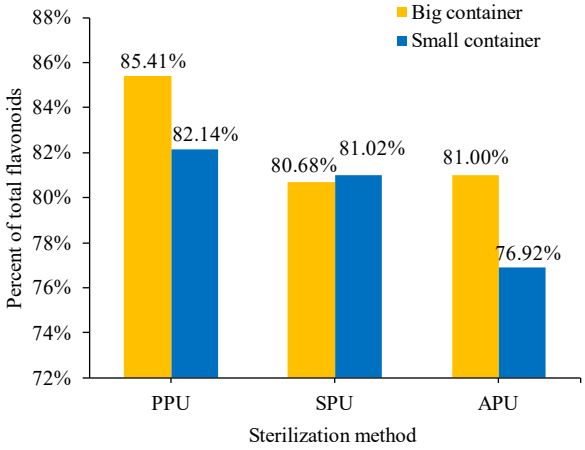

Figure 2. Comparison of total flavonoid content percentage after sterilization with pasteurization, steaming, and autoclaving. *($p<0.05$). Sterilization methods were applied for 15 minutes

The total flavonoid content in autoclaved extract (APU) significantly decreased. This result agrees with the study by Palma *et al.* [14], which explained that the decrease in TFC is related to the long exposure periods and the low thermal stability of flavonoids, as these compounds are destroyed in water at high temperatures. It is suspected that the size volume of the container affects the heat inside the container. The bigger the volume of the container, the more significant the surface area and the greater the air space. According to Boyle's law, if the temperature of a gas in a closed room is kept constant (isothermal), then the pressure of the gas will be inversely proportional to its volume [15, 16].

The relationship between container size and its impact on sterilization is noteworthy. Bigger containers inherently possess a greater surface area and a larger air space. This increased air space results in lower pressure within the container, leading to reduced heat levels. Conversely, smaller containers have a smaller surface area and air space, creating higher pressure and subsequently elevating the temperature within the container. Higher temperatures accelerate hydrolysis, potentially leading to the destruction of flavonoid compounds. This is why APU and SPU contain fewer flavonoid compounds compared to PPU. An odd observation arises from the SPU extract, which surprisingly exhibits a lower flavonoid content than the APU extract despite the sterilization temperature during steaming being lower than that in the autoclave.

3.2. Determination of Total Phenolic Content (TPC)

The total phenolic content of the extract was determined by Folin-Ciocalteu reagent in an alkaline atmosphere. The principle of this method is the formation of complex blue compounds that can be measured at a wavelength of 765 nm [17]. The principle of this method is the reduction of Folin-Ciocalteu reagent (FCR) in the presence of phenolics, resulting in the production of molybdenum–tungsten blue that is measured spectrophotometrically at 765 nm.

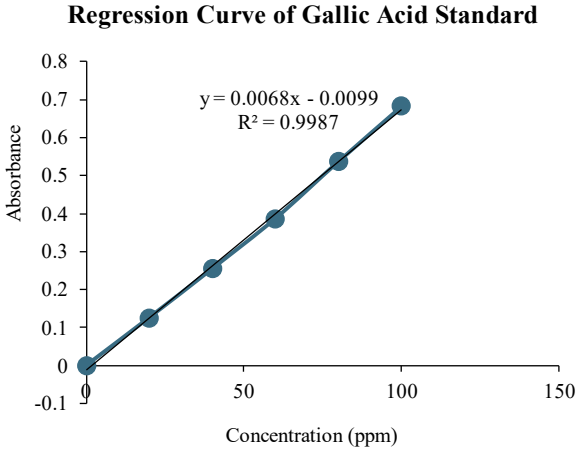

Figure 3. Regression curve of gallic acid standard

Determination of the total phenolic content of meniran's methanol extract was carried out using the Folin-Ciocalteau method with gallic acid as a standard solution because it is a stable and natural phenolic. The reaction between Folin Ciocalteau's reagent and gallic acid will form a yellow color, which indicates that there are phenolic compounds. A blue molybdenum-tungsten complex forms when phenolic compounds with hydroxyl groups react with Folin Ciocalteau [13]. The function of adding Na_2CO_3 solution is to provide an acidic atmosphere so that protons can dissociate into phenolic ions.

Based on testing, phenolics were only detected in meniran extract using methanol solvent. This phenomenon can be attributed to methanol's polar nature, which enables it to effectively dissolve phenolic compounds, which are also polar [17]. Phenolic content was determined at a wavelength of 765 nm according to the maximum Folin Ciocalteau wavelength using a UV- Vis spectrophotometer. The regression curve of the gallic acid standard solution is y=0.0068x – 0.0099 with R^2 = 0.9987, and it can be seen in Figure 3.

It can be seen that the concentration of the gallic acid standard solution is the same as the absorbance value. A higher standard gallic acid solution concentration corresponds to a greater absorbance value. This occurrence results from the increased production of molybdenum-tungsten complex products due to the reduction of phenolic ions by heteropolar acids. Consequently, this process generates a more intense color.

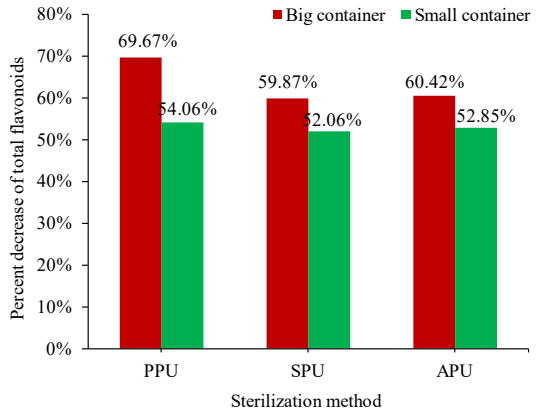

Figure 4. Comparison of remaining phenolic content percentage after sterilization with pasteurization, steaming, and autoclaving. *(p<0.05). Sterilization methods were applied for 15 minutes

Table 2 shows that the total phenolic content in *Phyllanthus urinaria* sterilized using all methods did differ markedly from those without sterilization (control). However, SPU and APU produced less phenolic content (p<0.05) than PU and PPU. Based on Figure 4, among the sterilization techniques, pasteurization in a big container resulted in remaining phenolic contents (69.67%).

The total phenolic content observed in the autoclaved extract (APU) significantly decreased. This outcome aligns with a study by Vergara-Salinas *et al.* [18], which reported similar findings in deodorized Thyme (*Thymus vulgaris*), where higher temperatures and prolonged exposure durations led to reduced phenolic compound yield. The sterilization process significantly impacted the phenolic compounds in *Phyllanthus urinaria* raw materials, as noted in the study by Pedrosa *et al.* [4]. This study concurs with previous research, which has shown variations in phenolic content in legumes, contingent upon the type of legume, conditions, and the methods employed for detection, as articulated by various authors.

Additionally, findings from research conducted by Maheshu *et al.* [19] indicated that pressure cooking reduced the total phenolic content by nearly 50% compared to its raw material state. This reduction is attributed to lixiviation, where phenols are drawn into the cooking water, and their binding with other compounds forms insoluble complexes [19]. For a more comprehensive elucidation, refer to the LC-MS analysis results.

3.3. Liquid Chromatography-Mass Spectrometry (LC-MS) analysis

The LC-MS analysis aims to ascertain the alterations in bioactive compounds resulting from sterilization. The samples used for this analysis were extracts from big containers, specifically from PU and APU. The selection criteria for the samples subjected to LC-MS analysis focuses on autoclaved extracts due to the prevalent use of autoclaves in biochemistry research. This choice was intended to offer insights into the compounds that might be lost or reduced following the sterilization process. The LC-MS profiles have successfully identified bioactive compounds in unsterilized (PU) and autoclave-sterilized *Phyllanthus urinaria* (APU) based on chemotaxonomy, as delineated in Table 3.

Table 2. The average total phenol content (mgGAE/g extract) of *Phyllanthus urinaria* after sterilization with different methods

Container's volume	Sterilization method			
	PU (control)	PPU	SPU	APU
Big	38.02±0.190	26.49±0.591*	22.77±0.230*	22.97±0.155*
Small	38.02±0.039	20.56±0.093*	19.79±0.295*	20.09±0.124*

Values are mean ± SD of duplo tests; (*) means significantly different (p<0.05);
PU: Control/extract without sterilization
PPU: Pasteurization-sterilized extract
SPU: Steaming-sterilized extract
APU: Autoclave-sterilized extract

Figure 5 shows that many metabolite compounds appear in the LC-MS chromatograms of the PU sample compared to the LC-MS chromatograms of the APU sample. Figure 6 shows that several metabolite compounds disappeared, possibly due to thermal hydrolysis. LC-MS analysis of *Phyllanthus urinaria* methanol extract between PU and APU showed differences in the composition of phenolic compounds after autoclaving. The data obtained from LC-MS also highlighted the loss of two compounds following the autoclaving process: ρ- hydroxybenzaldehyde (a phenolic compound) and naringenin (a flavonoid). The concentration of metabolites in APU decreased compared to PU. This result is in accordance with the previous results of determining the total content of TFC and TPC, which stated that the content of TFC and TPC was reduced compared to unsterilized extracts (control).

The phenolic tentatively identified in PU was suspected of having a similar structure as ρ- hydroxybenzaldehyde. Interestingly, ρ-hydroxy-benzaldehyde was not detected in APU. This can be explained by a lixiviation phenomenon that drives phenols into cooking water and by their being bound to other compounds, forming insoluble complexes [19]. ρ- hydroxybenzaldehyde is an example of a phenolic with tremendous pharmacological roles. Structurally, ρ- hydroxybenzaldehyde has a phenolic group with aldehyde on para-position that has more activity, such as anti-inflammatory, anti-angiogenic, antioxidant, and anti-diabetic [20].

On the other side, gallic acid increases in APU. This may be attributed to the hydrolysis of tannin by hydrothermal into gallic acid, as it was seen to be present in APU in a considerable amount. All of the *Phyllanthus urinaria* tannins are hydrolyzable tannins [1]. Hydrolysable tannins include gallotannins, ellagitannins, and phlorotannins, which release gallic acid, ellagic acid, and phloroglucinol upon hydrolysis (Figure 7) [21]. Furthermore, methyl brevifolincarboxylate and ethyl gallate (Table 3) decreased in APU.

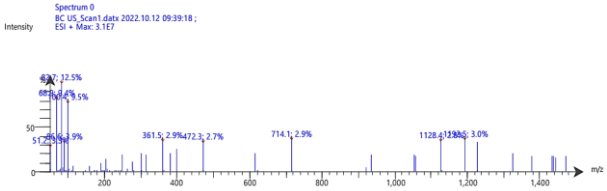

Figure 5. LC-MS chromatogram of PU (control)

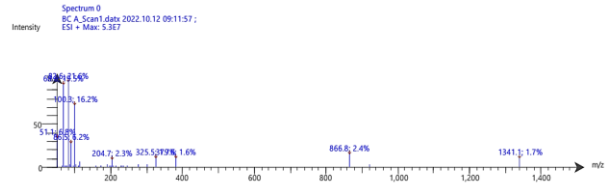

Figure 6. LC-MS chromatogram of APU (autoclaved)

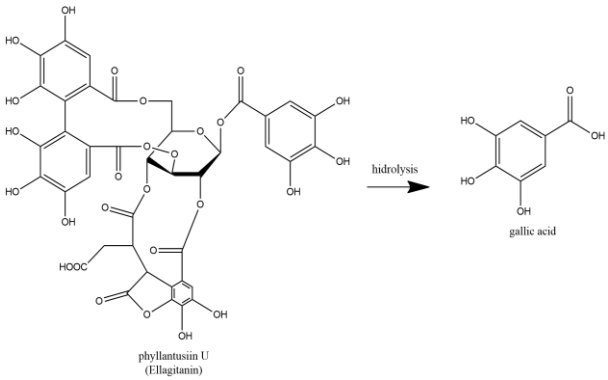

Figure 7. *Phyllanthusiin U* (ellagitannins) yield gallic acid. Although *Phyllanthus urinaria*'s tannin fractions yield gallic acid after hydrolysis, the parent compounds remain unidentified

Table 3. LC-MS profile of PU and APU of *Phyllanthus urinaria*

Bioactive compounds	m/z		Peak area	
	Theoretical	Experimental	PU (control)	APU
ρ-Hydroxybenzaldehyde [1]	122.123	120.8	2.6E+10	-
Naringenin	272.25	274	8.7E+04	-
Quercetin [22]	302.23	302	-	3.2E+06
Rutin [23]	610.517	610	6.2E+06	3.1E+06
Kaempferol [24]	286.23	286	6.3E+06	6.6E+06
(+)-Cucurbic acid [25]	212.28	212	2.0E+05	3.4E+05
Gallic acid [23]	170.12	167.4	8.8E+10	1.6E+11
Methyl brevifolincarboxylate [24, 26]	306.22	309	2.6E+05	2.0E+05
Ethyl gallate [27]	198.17	200	1.1E+04	1.0E+04

The flavonoid tentatively identified in PU was suspected to have a similar structure as naringenin (Table 3). However, naringenin was not detected in APU in this study. Previous research has also reported that naringenin was absent in *Phyllanthus urinaria*, which may be due to different environmental conditions. Behdad *et al.* [28] explained that different environmental conditions can dramatically influence the production of secondary metabolites in *Glycyrrhiza glabra* [29]. The absence of naringenin is in accordance with the TFC results, which showed a significant decrease in flavonoid content in *Phyllanthus urinaria* after autoclaving (APU).

Moreover, the flavonoid was found to be present in APU, which was suspected to have a similar structure as quercetin (Table 3). However, quercetin was seen to be absent in PU. This may be explained by the hydrothermal degradation of rutin to quercetin [29], which, consequently, quercetin only appears in APU. Furthermore, rutin, a flavonoid glucoside, had a reduced peak in APU, indicating that rutin has been transformed into a smaller molecule, decreasing its content. This result is in accordance with the TFC result, in which there was a significant decrease in flavonoid content in the *Phyllanthus urinaria* after autoclaving (APU). It is known that high temperatures provoke polymerization and decomposition in the structure of aromatic rings of polyphenols, making their quantification difficult. Contact with water at high temperatures could increase the solubility of polyphenols, thereby increasing their release into the cooking water. As more water volume was used for cooking, a higher loss of polyphenols was observed [30].

Another study confirmed that the model glycoside compound quercetin-3-O-rutinoside (rutin) has been subjected to subcritical water within the temperature range of 120 to 220°C. The hydrothermal degradation products showed rutin degradation to quercetin [29]. Kaempferol was detectable in both PU and APU, yet the peak area of kaempferol displayed an augmentation in APU, signifying an escalated concentration of kaempferol following autoclaving. This change could be elucidated by the partial hydrolysis of hemicellulose during a water-based thermal process, liberating phenolic compounds associated with the cell wall. Therefore, the presence of these compounds in APU is likely connected to the hydrolysis of cell wall polysaccharides, leading to the release of additional compounds [18, 31].

An organic acid was present in PU and APU, which was suspected of having a structure similar to (+)- cucurbic acid (Table 3). The peak area of (+)-cucurbic acid was seen to increase in APU. This may be attributed to the water-based thermal process; part of the hemicellulose is hydrolyzed and forms acids. These acids are assumed to catalyze the hydrolysis of remaining hemicelluloses [32]. (+)-cucurbic acid is a hydroxy monocarboxylic acid. It has a role as a member of jasmonate derivatives [25]. This compound emitted by plants has important functions in plant communications, direct and indirect defenses, and protection against abiotic stresses [33].

4. Conclusion

This study concludes that the pasteurization process at 70°C for 15 minutes is the most favorable method for sterilizing meniran herbal (*Phyllanthus urinaria*). This method minimizes the adverse impact on phytochemical compounds and helps preserve the total flavonoid and phenolic content. LC-MS profile showed that APU loss of the ρ-hydroxybenzaldehyde and naringenin compound. Furthermore, APU decreased rutin, methyl brevifolincarboxylate, and ethyl gallate content. Hence, the pasteurization method could be used as an alternative for sterilizing *Phyllanthus urinaria*.

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