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*Article*

# Extraction of Gallic Acid from *Chromolaena sp.* Using Ultrasound-Assisted Extraction

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**Abstract.** *Chromolaena sp.* is believed to have phytochemical components namely alkaloids, flavonoids, flavone, essential oils, phenolics, saponins, tannins and terpenoids. In this study, ultrasound-assisted extraction (UAE) procedure was performed to extract the gallic acid from *Chromolaena sp.* UAE is known to be an environmentally green extraction method. This study was carried out with two different parameters which are sonication time and duty cycle. Phytochemical screening result showed the presence of phenolic compound when the dark-green colour of solution was observed. The best operating parameters to maximise the yield were as follows: sonication time of 80 minutes with yield of 3.006 mg/mL and duty cycle of 90% with yield of 3.764 mg/mL. The FT-IR result shows that presence of O-H and alkene group in the extraction samples. From the results, it can be concluded that UAE is an effective method to extract gallic acid from *Chromolaena sp.* The implication in this study was reducing the extraction time for the production of herbs medicine from natural resource.

**Keywords:** Phytochemical components, Gallic acid, Ultrasound-Assisted Extraction (UAE), Sonication time, duty cycle.

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## 1. Introduction

Phytochemical plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, phenols, steroids and flavonoids. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro [1].

Phenolic compounds from the phytochemical plant have been a popular research topic due to their importance characteristic in the health and nutrition fields. Their common advantages are high antioxidant activity and free radical scavenging capacities produced in metabolic processes. These properties help to fight against cancer, cardiovascular diseases, chronic diseases, and so forth. For example, flavonoids, polysaccharides, and phenolic acids, especially gallic acid, are widely applied in beverages, cosmetics, food, and medicine as functional products [2].

Gallic acid is one of the members of phenolic compounds and has been described to have the properties of anti-inflammatory, anti-mutagenic, anti-oxidative, anti-free radical, and other bioactive properties. Furthermore, gallic acid also has antitumor effect that preventing metastasis of mast cell tumours, thereby extend and increase the survival period, and is a relatively suitable trypanocidal drug candidate and has a great defensive effect on the liver [3].Grapes, tea, and other plants are the example of commonly producer for the naturally polyphenolic compound such as gallic acid. The gallic acid primarily produced by several ways such as acid hydrolysis, alkaline hydrolysis, fermentation, and enzymatic hydrolysis, but these methods have their different disadvantages, for example, critical acid corrosion of the equipment, difficult process, long reaction period, and incomplete hydrolysis. Therefore, it is quite important to find friendly and green solvents process.

At current time, the most practiced sample extraction methods including ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), reflux extraction (RE), enzymatic extraction (EE), and supercritical fluid extraction (SCFE) have been a common ways to extract the active compounds from the natural plants. Among several extraction techniques, UAE has recently become a very important technique in sample preparation and extraction of active components because it has the significant advantages of high efficiency, fast effect, energy-saving, and minimum solvent consumption [2] which are indicated to green and friendly process. By using the organic solvents in the UAE method was also highlighted the need to enhance the 'green chemistry' trend [3].

The UAE is also considered to have a great potential application in extraction field. Using ultrasound at the frequency of 20-100 kHz, extraction process processes can now be completed in seconds or minutes with high reproducibility, simplifying operation and work-up scale, removing post-treatment of waste water, reducing solvent intake and energy input. As an extraction technology, UAE concern much more attention and is widely used in the fields of separation and extraction in recent years. The extraction of antioxidant compounds, phillyrin, polysaccharides, polyphenols, eight ginsenosides, flavonoids, betulin, vanillin, phenolics, carotenoids, etc has been successfully informed in the extraction of natural product [4].

In this study, *Chromolaena sp.* as a medical plant was chosen to extract the gallic acid by using UAE method. Chromolaena odorata (Family: Asteraceae) synonyms as Eupatorium odoratum is a traditional medicinal plant that is commonly used for its wound healing properties. In explanation, several parts of this herb have been applied to heal wounds, burns, and skin infections [5]. Previous research by Sukanya et al. [6] show that the leaves and stems of Chromolaena odorata presence of essential oils, steroids, triterpenes, alkaloids, phenolic and flavonoids by using solvent based extraction method.

In this study, the main significant chemical compound that needed to be extracted was phenolic compound in a form of gallic acid by using UAE as extraction method. Study of single parameter such as sonication time and duty cycle was investigated to study the best operating parameter for the extraction of gallic acid from *Chromolaena sp.* by using UAE method. Characterization of *Chromolaena sp.* extract was determined by using FTIR to study the functional group of *Chromolaena sp.* extraction. Finally, extraction of gallic acid from *Chromolaena sp.* by using UAE method expected to be an effective method and produce high concentration of gallic acid.

## 2. Materials and Methods

### 2.1. Materials and Chemicals

*Chromolaena sp.* was collected from Kudang Hill, Jeli, Kelantan, Malaysia. The whole plant was use in the extraction. Samples were air dried ground and passed through 300 mesh screen.

The chemical that being used in this study were distilled water, Iron (III) Chloride ($FeCl_3$), gallic acid standard from Merck Sdn Bhd., water (HPLC grade), acetic acid (HPLC grade) and methanol (HPLC grade).

### 2.2. Methods

#### 2.2.1. Instrument

The extraction process of pre prepared *Chromolaena sp.* was performed by using ultrasound (Q Soniqa) working at 60 amplitude and input power of 40-120 W. HPLC analysis was performed using Perkin Elmer series 200 by using HPLC- DAD (diode array detector). The

separation achieved by a reverse phase C-18 column. Gradient elution of mobile phase that comprised a mixture of two solvents: 1% Acetic acid in water and methanol. The gradient program was started with 30, 60 and 90% of B at 2, 4, and 6 minute respectively. The wavelengths detection was read at 250 nm and 360 nm using a flow rate of 1.2 mL/min and sample injection volume of 50 µl. The stock solution of Gallic Acid was prepared and further diluted with the mobile phase solvent for standard curve. FTIR analysis was studied by analysing the prepared standard of gallic acid then followed by the sample. FTIR used in this study was the ATR model (Spectrum 400 FT-IR, Perkin Elmer). The wavenumber range was set between 4000-650 cm-1. Two drops of samples was placed on the surface of the sample holder to be analysed. The analysing procedure for sample was same as standard.

2.2.2. UAE Procedure

The extraction method started with the 10 g sample was placed into 100 mL of distilled water inside a 250 mL beaker. Then, it was stirred using a spatula until the mixture fully mixed. The sample was then placed in the ultrasound sonicator at 20, 40, 60, 80 minutes for the parameters of sonication time. And 90, 75, 60, 45% for the duty cycle parameters. Both of the parameters were repeated for 3 times each. After all the parameters were done with the sonication process, it was then transferred into a 250 mL centrifuge bottle to be centrifuged with temperature of 21◦C for 30 minutes with 5800 rpm. The supernatant was filtered, collected and stored at 4◦C. The samples then undergo several analyses such as FTIR and HPLC.

2.2.3. Phytochemical Screening Test

Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture and an important tool in bioactive compound analysis. In this screening process, the sample was screened using Iron (III) Chloride ($FeCl_3$). 2 mL of the sample was placed in a test tube then; 2 drop of Iron (III) chloride was dropped to see the presence of the gallic acid. Changes of dark-green color of the sample were observed during the test [7, 8].

## 3. Results and Discussion

### 3.1. Phytochemical Screening Analysis

In order to implement the phytochemical screening, the test of gallic acid was performed by adding 2 mL of 5% aqueous iron (III) chloride into 2 mL of each extract. Based on the theory, by adding the iron (III) chloride into the extract solution, there will be formation of dark-green color and precipitation that indicates the formation of phenols in the sample extract [9]. From the Table 1, we can see that the presence of gallic acid is increasing with the sonication time. Thus, sonication time of 80 minutes has the highest amount of gallic acid. The pattern was similar with duty cycle as parameter. Increasing duty cycle result a darker green color produced. Thus, the highest amount of garlic acid was predicted by using 90 % of duty cycle.

Table 1. Phytochemical screening analysis for *Chromolaena sp.*

| | Effect of Parameter | | | Presence of Gallic acid |
|---|---|---|---|---|
| 1. | Sonication time | 20 min | Duty Cycle: 75% | + |
| 2. | | 40 min | | ++ |
| 3. | | 60 min | | ++ |
| 4. | | 80 min | | +++ |
| 5. | Duty Cycle | 90 % | Sonication time: 40 min | +++ |
| 6. | | 75 % | | ++ |
| 7. | | 60 % | | ++ |
| 8. | | 45 % | | + |

Note: (-): Absence of turbidity/ flocculation/ precipitation
(+): Slight opacity
(++): If the reactive product and not turbidity flocculation
(+++): Present of precipitate/ flocculation heavy
*All must in dark-green colour

### 3.2. Study of Single Parameter

In this study, ultrasound-assisted extraction (UAE) was conducted using two parameters to optimize the extraction yield. The parameters studied were duty cycle and sonication time. The duty cycle was done by several values which were 90, 75, 60, 45%. Meanwhile, the sonication time was run by 20, 40, 60, 80 minutes for each sample. Each extraction at certain operating parameter sample was conducted for three times.

3.2.1. Effect of Duty Cycle Parameter on the Yield of Gallic Acid

The yield of gallic acid by different duty cycle percentage during the sonication method from 90% to 45% was shown in Table 2 and Fig. 1. The constant parameters were as follows: frequency 60 Hz, power 500 watt, sonication time 40 minutes and amplitude 60%. The retention time of gallic acid sample was detected at 2.397 min. The result indicated that the maximum concentration of gallic acid was reached when the duty cycle is 90% and began to decrease at 75 %. This might be happened due to decrease the duty cycle also decrease the contact surface area between the solvent and targeted compounds [10, 11]. 90% duty cycle means, in one minute cycle of sonication process, 6 second off and 54 second on. That's means, when the percentage of the duty cycle on sonication process is high; the contact of the sonicator probe with the sample was increased,

resulted high concentration of gallic acid. As we can see, the highest percentage of duty cycle has the highest amount of gallic acid concentration which is 3.764 ± 0.083 mg/mL at 90% duty cycle.

In UAE process, acoustic cavitation and some mechanical effects resulted from the ultrasound can enhance the extraction efficiency. Zhang et al. [12] and Yuhai et al. [11] point up the disruption of cell wall by acoustic cavitation and facilitating the solvent to penetrate into plant material and allowing the intracellular product release. Agitation of solvent used for the extraction is one of the mechanical effects of ultrasound. This effect can increase the contact surface area between the solvent and targeted compounds by permitting greater penetration of solvent into sample matrix [11, 12]. So, it can be concluded that at highest percentage of duty cycle, the process of acoustic cavitation and some mechanical effect occur more often compared to other duty cycle percentage during the process of sonication in order to break the cell wall. Previous study of extraction gallic acid using UAE method from Labisa Pumila Sp. [13] also showed the same pattern. From the variations of duty cycle tested for the extract of gallic acid from Labisa Pumila sp., 50% duty cycle was proven to perform a better result of gallic acid yield. In 25 L mobile extractor, by using 50% duty cycle and 8 hours sonication time resulted 133.250 mg/g extraction yield.

Table 2. Yield of gallic acid based on effect of duty cycle parameter.

| No. of sample | Duty cycle (%) | Yield (mg/mL) | Standard deviation |
|---|---|---|---|
| 1 | 90 | 3.764 | 0.083 |
| 2 | 75 | 2.985 | 0.014 |
| 3 | 60 | 1.905 | 0.087 |
| 4 | 45 | 1.038 | 0.081 |

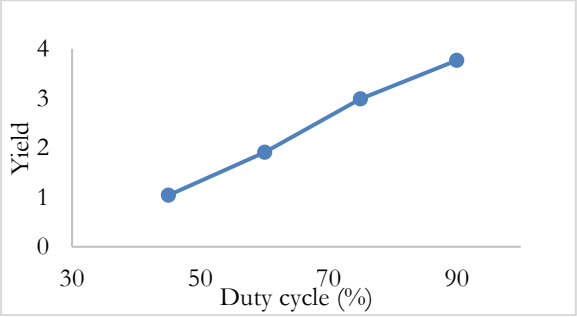


Fig. 1. Yield of gallic acid from *Chromolaena sp.* extraction using duty cycle as parameter.

3.2.2. Effect of Sonication Time Parameter on the Yield of Gallic Acid

The effect of sonication time on extraction yield of gallic acid was shown in Table 3 and Fig. 2. The extraction time was set at 20, 40, 60, 80 minutes to determine the effect on the yield of gallic acid. Sonication process releases energy and heat. Increasing the extraction time more than 80 minutes may lead to the temperature rises and affect the bioactive compound. Certain bioactive compound such as gallic acid may mobilize and decomposed at higher temperature [14]. The constant parameters for extraction of gallic acid from *Chromolaena sp.* by using UAE were frequency 60 Hz, power 500 watt, duty cycle 75% and amplitude 60%. The concentration of gallic acid increased with the increasing sonication time and reached the maximum value 3.006±0.065 mg/mL at 80 minutes. The same pattern was obtained from the previous research by Liu et al [8]. From this previous research, the yield of polysaccharides from lyceum was increased by increasing the sonication time. This phenomenon can be explained by the more time contact of sonication probe with the sample (sonication time), the more cell are broken down thus resulting more yield of gallic acid produced. The lowest gallic acid concentration produced from 20 min of sonication time result 0.559±0.045 mg/mL yield of gallic acid. This results can be explained by the acoustic cavitation and some mechanical effects resulted from the ultrasound-assisted extraction can enhance the extraction efficiency. The concept of acoustic cavitation and mechanical effect on the cell wall of the sample was the same with duty cycle. In other words, at highest sonication time, the process of acoustic cavitation and some mechanical effect occur more often compared to other sonication time during the process of sonication in order to break the cell wall. This finding support previous research that the sonication time was a significant factor in the UAE of gallic acid from Labisa pumila [13] and Aspergillus niger [15]. Chysirichote and Pakaweerachat [15] also compared the yield from the water extraction without ultrasound assistance and with ultrasound assisted. The results showed that using the ultrasonic assistance increased the extraction yield of gallic acid from 0.25±0.03 to 1.26±0.25 g/g with the shorter extraction time from 60 min to 30 min.

Table 3. Yield of gallic acid based on effect of sonication time parameter.

| No. of sample | Sonication time (min) | Yield (mg/mL) | Standard deviation |
|---|---|---|---|
| 1 | 20 | 0.559 | 0.0458 |
| 2 | 40 | 1.694 | 0.121 |
| 3 | 60 | 2.722 | 0.09 |
| 4 | 80 | 3.006 | 0.065 |

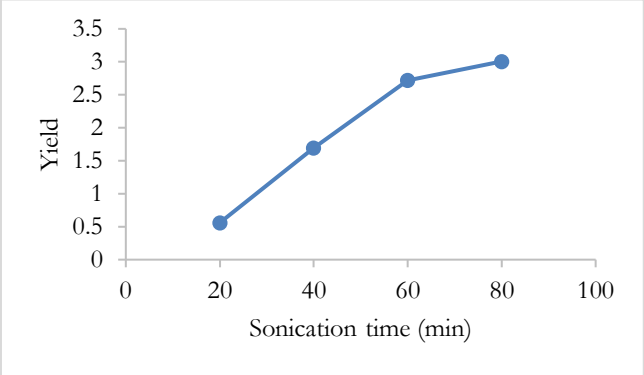


Fig. 2. Yield of gallic acid from *Chromolaena sp.* extraction using sonication time as parameter.

### 3.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrum was used to identify the functional groups of the active compounds represent in the extract based on the peaks values in the region of IR radiation. It happens when the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio [16]. The IR spectra of sample were basically indistinguishable only with some difference of bands with standard. This result may be due to the fact that the standard is a neutral gallic acid while for the sample are acidic gallic acid. However, both of the peaks detected in standard (Fig. 3) and samples (Fig. 4 & Fig. 5) were sufficient to enable spectral identification from FT-IR data. The infrared spectroscopy in all IR figures shows stretch vibration of O-H. The alkene group existed in standard of gallic acid was proved by the stretch vibration of C=C at around 1636 $cm^{-1}$ corresponding to carbon-carbon double bond asymmetric stretching vibration absorption area. The absorption band within range of 3600-3300 $cm^{-1}$ was the characteristic of gallic acid. No significant differences can be seen in the fingerprint area of standard sample with the samples. The results of FT-IR analysis confirmed the presence of phenol and alkene. This result was accomplished by referring to the previous study by Yin *et al.* [17].

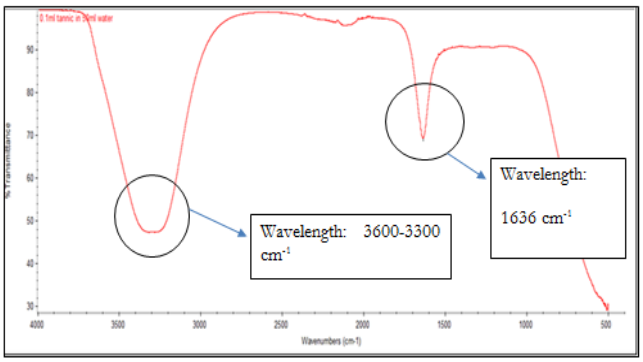


Fig. 3. FT-IR spectra for standard gallic acid.

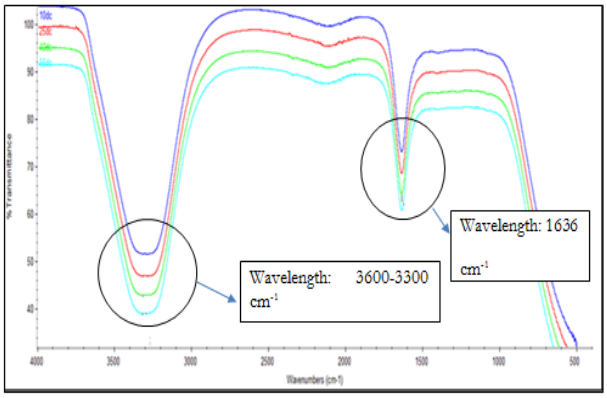


Fig. 4. FT-IR spectra for duty cycle parameter for extraction of gallic acid.
Note: blue navy line, red line, green line and aqua blue line are projected duty cyle of 90%, 75%, 60% and 45%, respectively.

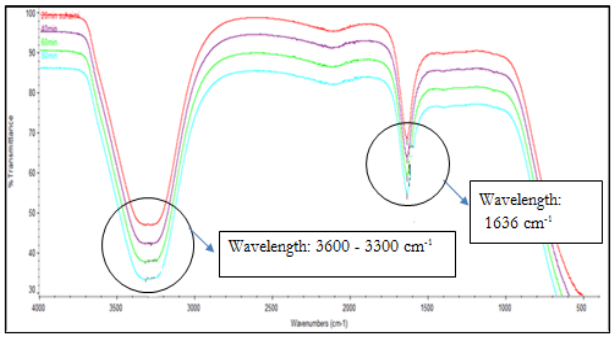


Fig. 5. FT-IR spectra for sonication time parameter for extraction of gallic acid.
Note: red line, purple line, green line and aqua blue line are projected sonication time of 20, 40, 60 and 80 min, respectively

## 4. Conclusions

In this study, Gallic acid was extract from *Chromolaena sp.* by using ultrasound-assisted extraction (UAE) method. UAE method is well known as a more environmentaly friendly methods by decreasing the solvent usage, reducing the extraction time, increasing the extraction yield and enhancing the quality of extract during the extraction. From this study, the UAE method is proven to be a good method to extract gallic acid from *Chromolaena sp.* In summary, three primary results are presented in this study. The phytochemical screening of gallic acid by using Iron (III) Chloride shows the presence of gallic acid when the dark-green colour of the solution was observed. The single parameter studies which are sonication time and duty cycle observed in this ultrasound-assisted extraction (UAE) in order to extract gallic acid. Form the result, the best operating condition of gallic acid extraction by using UAE method was 80 minutes of sonication time with the concentration of 3.0060 mg/mL and 3.7641 mg/mL of concentration obtained from the 90% of duty cycle parameter. Fourier transform infrared spectroscopy (FT-IR) was used to determine the functional groups that exist in the standard

and samples. The FT-IR result shows that there is O-H group at range of 3600-3300 $cm^{-1}$ and alkene group (C=C) at range 1636 $cm^{-1}$.

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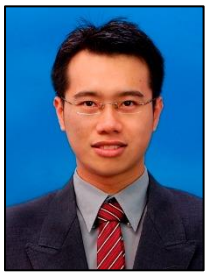

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