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Effects of Extraction Method on Yield, Phenolic and Flavonoid Content of Leaf, Stem and Root of *Cassia alata* Linn.

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ABSTRACT

The study of medicinal plants has gained significant interest among researchers because of their potential for therapeutic purposes and the production of natural drugs. In Sarawak, *Cassia alata* is one of the native plants used for medicinal purposes, such as treatment for constipation, ringworm, and other skin diseases. This study determined the yield of extraction, total phenolic content (TPC), and total flavonoid content (TFC) of the leaf, stem, and root of *C. alata* using various extraction methods and solvent extractions. The extractions were performed using soxhlet extraction (SE) and ultrasonic-assisted extraction (UAE) with ethanol and chloroform. Among all, the extract obtained from SE with ethanol solvent (SE-EtOH) showed the highest yield in all plant parts (leaf: 28.62 %, stem: 10.06 %, and root: 9.79 %). Meanwhile, the TPC and TFC estimated using the Folin-Ciocalteu phenol reagent and aluminium chloride colorimetric assay methods showed that the highest TPC and TFC were from the leaf extract obtained using UAE and chloroform (UAE-Chlo-L) with a TPC value of 117.436 mg GAE/g DW and a TFC value of 568.778 mg QE/g DW, respectively. Overall, the findings demonstrated that chloroform was an effective solvent system for all plant parts on the TPC and TFC, with the leaf part containing the greatest value, and that ultrasonic-assisted extraction was the best approach. This exploration is beneficial for the determination of methods that produce optimum yield, phenolic, and flavonoid content in *C. alata*'s species.

Keywords: *Cassia alata*, TPC, TFC, solvent system, soxhlet extraction, ultrasonic assisted extraction

INTRODUCTION

Plants possess a wide range of organic substances that are classified as primary and secondary metabolites. Primary metabolites such as phytosterols, acyl lipids, nucleotides, amino acids, and organic acids are fundamental for photosynthesis, reproduction, respiration, growth, and development (Wang *et al.*, 2022). Secondary metabolites are molecules that help plants interact with their surroundings (Adedeji & Babalola, 2020). Phenolics, flavonoids, alkaloids, terpenes, saponins, lipids and carbohydrates are the classes of secondary metabolites (Hussein & El-Anssary, 2019). The determination of secondary metabolites in plant extracts is important as it helps in supporting the development of modern medicines and supplements. According to Rahman *et al.*, (2022), the determination of total phenolic content (TPC) and total flavonoid content (TFC) are significant parameters to evaluate the potential health benefits of plant extracts such as antimicrobial, antioxidant and anti-inflammatory activity.

Phenolics are secondary plant metabolites with over 8000 structures, including lignans, tannins, phenolic acids, stilbenes, and flavonoids. They are commonly found in plant tissues such as in fruits, seeds, leaves, stems and roots. These phenolics are associated with various health benefits, such as anti-ageing (Dhalaria *et al*., 2020), anti-proliferative activities, anti-inflammatory and antioxidant properties (Cianciosi *et al*., 2018; Cardoso *et al*., 2020). The flavonoid, on the other hand, is a member of a group of polyphenolic-structured secondary plant metabolites. It has a wide range of biological effects, including hepatoprotective, anti-inflammatory (Jiang *et al*., 2019), antibacterial, antioxidant (Li *et al*., 2019), and anti-hyperlipidemic effects (Bencheikh *et al*., 2021).

Cassia alata, also called *Senna alata*, is one

of the native plants in the Leguminosae family with many medicinal uses. Its local name in Malaysia is "*pokok gelenggang*" (Mat Jusoh *et al*., 2023). In the Sarawak community, the leaves of *C. alata* have been used to treat constipation, ringworm, and other skin diseases by decoction and pounding (Bakar *et al*., 2023). Meanwhile, in Indonesia, the roots are used by the Dayak tribe to treat ringworm diseases (Az-Zahra *et al*., 2021). According to Fatmawati *et al.* (2020), *C. alata* has various pharmacological activities such as antibacterial, antidiabetic, anti-inflammatory, antifungal and antioxidant. For example, the ethanolic extracts from the leaves were suggested as potential active components for skin care products due to their antioxidant and anti-inflammatory properties (Saidin *et al.*, 2019). Besides, the bark and roots also showed antibacterial activity against *Salmonella enterica* serovar Typhimurium, *Staphylococcus aureus*, and *Escherichia coli* (Halim-Lim *et al.*, 2020). The various ethnomedicinal benefits of *C. alata* have prompted researchers to analyse plant sources for bioactive compounds. This approach is significant for developing new pharmaceutical and biological resources in healthcare systems (Vaou *et al*., 2021).

There is scant literature reported on the bioactive compounds of *C. alata* using different extraction methods and solvents (Oladeji *et al.*, 2020). Recently, researchers have gained interest in the application of soxhlet extraction (SE) and ultrasonic assisted extraction (UAE) using various solvents to extract secondary metabolites from plants (Ling *et al*., 2019). SE also known as "hot continuous extraction," is a traditional technique that is often used in small research settings. This process has significant exposure to dangerous and combustible organic solvents and uses a high amount of solvent, time, and energy (Thilakarathna *et al*., 2023). Meanwhile, the UAE uses the sonic cavitation effect of ultrasound to improve the surface contact between samples, solvents, and cell walls. Unlike SE, UAE is an advanced method that can reduce time and solvent consumption (Deng *et al*., 2022).

Therefore, the evaluation of different extraction methods and solvents is crucial in finding an optimum yield, phenolic and flavonoid content specifically in *C. alata*'s species. In this study, the yield, TPC and TFC of leaf, stem, and root extracts of *C. alata* were determined using soxhlet and ultrasonic assisted extraction. Subsequently, the extracts obtained from ethanol extraction were compared with those obtained from chloroform extraction. These results are significant for assessing the efficacy of the methods in determining the quantity of phenolic and flavonoid in *C. alata's* species.

MATERIALS AND METHODS

Materials

The chemicals used were ethanol (HmbG, Malaysia), chloroform (Merck, Germany), Folin-Ciocalteu reagent (Sigma Aldrich, USA), gallic acid solution (HiMedia, India), sodium carbonate (Na_2CO_3) solution (Merck, Germany), quercetin solution (Sigma Aldrich, USA), sodium nitrate ($NaNO_3$) solution (Merck, Germany), aluminium chloride ($AlCl_3$) solution (Merck, Germany), and sodium hydroxide (NaOH) solution (Merck, Germany).

Preparation of *C. alata* Samples

The *C. alata* plants were collected from the Kota Samarahan roadside (1˚27'40.968" N, 110˚24'50.5" E). The plants were identified by the flowers, which resemble a fluorescent yellow candle and grow in a vertical column from the plant (Oladeji *et al*., 2020). The collected plant samples were separated into leaf, stem, and root parts. They were properly cleaned before being chopped into small pieces, dried in an oven at 40 °C for 5 days to achieve constant weight and ground into a powder (Mugao *et al*., 2020).

Extraction of *C. alata* Samples

In SE, approximately 15 g of the samples were weighed and put in a thimble of a soxhlet apparatus containing 200 ml of extraction solvent and extracted for 6 hr (Mahyuddin *et al*., 2020). Meanwhile, in UAE, about 1 g of samples were put in a Schott bottle containing 40 ml of extraction solvent and extracted for 30 min using an ultrasonic water bath (Saifullah *et al*., 2020). The extraction solvents used were ethanol and chloroform.

Determination of Extraction Yield

After the extraction, the extracts were filtered through Whatman No. 1 filter paper, vacuum-

dried with a rotary evaporator and left to dry completely in the fume hood. The yield of extraction (%) was calculated as follows; Eq.(1):

Yield of extraction (%) = $W_0 / W_1 \times 100$ Eq.(1)

Where W_0 and W_1 are the weight of *C. alata* extracted from the sample (g) and the weight of the sample (g), respectively. To avoid any potential degradation, the resultant dried crude extracts were packed and kept at 4 ˚C.

Determination of Total Phenolic Content (TPC)

The TPC of *C. alata* extracts was determined using the Folin-Ciocalteu reagent and gallic acid as a standard solution (Ling *et al.*, 2019). Briefly, 0.125 ml of the extract was put into a test tube along with 0.5 ml of ultrapure water and 0.125 ml of Folin-Ciocalteu phenol reagent. After 3 min, 1.25 ml of 7% Na_2CO_3 was added, and then distilled water was added to top up the volume to 3 ml. The absorbance was measured at a wavelength of 760 nm using a Shimadzu UV-1900i spectrophotometer after 1 hr of incubation at room temperature and in a dark room. Figure 1 shows a calibration curve for gallic acid. The TPC of the extract was expressed as mg of gallic acid equivalent (GAE) per g of dry weight (mg GAE/ g DW) by comparing with the gallic acid calibration curve.

Determination of Total Flavonoid Content (TFC)

The TFC of *C. alata* extracts was determined using an aluminium chloride colourimetric assay and quercetin as the standard solution, according to Ling *et al.* (2019). About 4.8 ml of ultrapure water and 0.3 ml of a 5% $NaNO_3$ solution were added to a test tube after an aliquot of 0.2 ml of extract was added. After 5 min, 0.3 ml of 10% $AlCl_3$ was added, followed by 2 ml of 1M NaOH, and the remaining volume was brought to 10 ml with ultrapure water. The absorbance was determined at a wavelength of 414 nm. Figure 2 shows a quercetin calibration curve. The TFC of the extracts was expressed as mg quercetin equivalent per gram of dry weight (mg QE/ g DW) by comparing with the quercetin calibration curve.

Statistical Analysis

Results were expressed as mean ± standard deviation (n=3). Pearson correlation test was performed by using IBM SPSS Statistics 27 to determine the correlation between the TPC and TFC.

RESULTS AND DISCUSSION

Yield of Extraction

The extraction efficiency is significant as it can reduce the use of solvent volume, amount of dry sample, sampling time, energy costs, and extraction time per extraction (Zhang *et al.*, 2018). Table 1 shows the extraction yield of *C. alata.* The amount of extraction yield obtained increased from low polarity to high polarity of solvents as follows: chloroform > ethanol. The highest extraction yield was obtained by SE-EtOH, where the leaf at 28.62 ± 0.69% followed by the stem (10.06 ± 0.05%) and roots (9.79 ± 0.14%). In comparison, UAE-Chlo recorded a lower extraction yield for all parts of *C. alata*. Bui *et al.*, (2021) reported a similar trend in the extract yields of *Avicennia officinalis* leaf using ethanol (8.95 ± 0.69%) and chloroform (3.20 ± 0.57%).

Ethanol is a polar solvent which is particularly effective at extracting polar compounds, including a wide range of

Table 1. Extraction yield (%) of *C. alata* using SE and UAE

Extracts	Soxhlet extraction	Ultrasonic assisted extraction
EtOH-L	28.62 ± 0.69	11.80 ± 1.40
EtOH-S	10.06 ± 0.05	3.57 ± 0.59
EtOH-R	9.79 ± 0.14	2.00 ± 0.44
Chlo-L	8.50 ± 0.34	4.93 ± 0.42
Chlo-S	1.14 ± 0.34	0.83 ± 0.12
Chlo-R	1.30 ± 0.56	0.73 ± 0.12

phytochemicals such as flavonoids and alkaloids (Alara *et al.*, 2021). Meanwhile, chloroform, being a non-polar solvent is highly effective at extracting non-polar compounds such as lipids (Saini *et al.*, 2021). A greater yield in ethanol extracts showed that the presence of polar compounds was higher in *C. alata*. This is because polar compounds such as polar carbohydrates and glycosides are readily dissolved in polar solvents such as ethanol (Bitwell *et al.*, 2023). Similarly, Dirar *et al.*, (2019) reported that most of the components in *C. alata* are hydrophilic and water-soluble components. Therefore, ethanolic extracts showed a greater extraction yield of crude extracts than chloroform extracts.

As for plant parts, the leaf of *C. alata* showed a greater yield of crude extract than other plant parts. Similar to Halim-Lim *et al*. (2020), using 95% ethanol, the leaf extract (32.17%) of *C. alata* showed a higher yield than bark (30.53%) and root parts (30.05%). This is probably because leaves are rich in bioactive compounds responsible for protection against environmental stress factors such as UV radiation (Aguirre-Becerra *et al*., 2021). Meanwhile, for the extraction method, SE yielded higher yields compared to UAE, regardless of solvent extraction and plant part used. Similar to Das *et al.* (2019), the leaves of *Piper betle*'s extract yield resulted in 11.00 for SE, 10.33% for maceration, and 8.00 for UAE. According to Mahyuddin *et al*. (2020), the soxhlet apparatus cycles the same solvent through the samples completely, which ensures the fresh solvent is continually used to dissolve the targeted compounds. Over time, this can result in a more complete extraction of compounds with limited solubility. Therefore, the SE showed a greater yield of extraction than UAE and all plant parts.

Total Phenolic and Flavonoid Contents of *C. alata*

The total phenolic content (TPC) in *C. alata* was determined using Folic-Ciocalteu reagent. Meanwhile, an aluminium chloride colourimetric assay was used to determine the total flavonoid content TFC. As shown in Figure 1, the TPC was expressed in mg GAE/g DW and obtained from the calibration curve of gallic acid. For total flavonoid content of each extract was expressed in mg QE/g DW and obtained from the calibration curve of quercetin (Figure 2). The Pearson correlation coefficient was performed to show the strength and direction of the linear relationship of correlation. The Pearson correlation between TPC and TFC in this study was $r = 0.949$ (p-value < 0.01). This showed a positive correlation between TPC and TFC.

In this study, leaf extracts showed greater phenolic content, regardless of the extraction techniques and solvent used. The highest was obtained from the leaves part, extracted using UAE and chloroform (UAE-Chlo-L, 117.436 mg GAE/g DW) (Figure 3). Similar to TPC, the results presented in Figure 4 showed that the extracts from the leaves, extracted using UAE and chloroform had a significantly high flavonoid content (UAE-Chlo-L, 568.778 mg QE/g DW). The results showed that different parts of plants had different values of phenolic and flavonoid compounds.

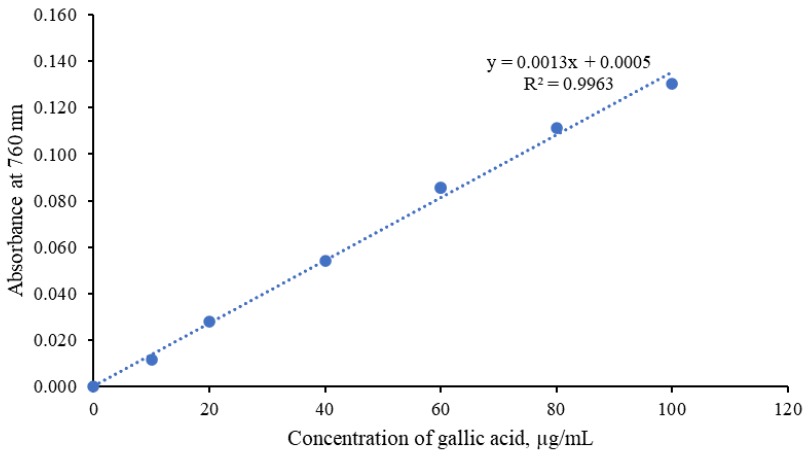

Figure 1. Gallic acid calibration curve

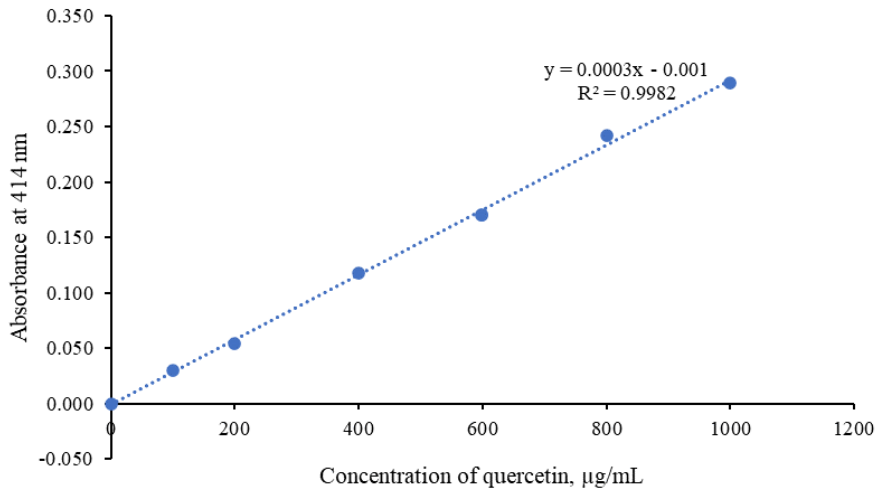

Figure 2. Quercetin calibration curve

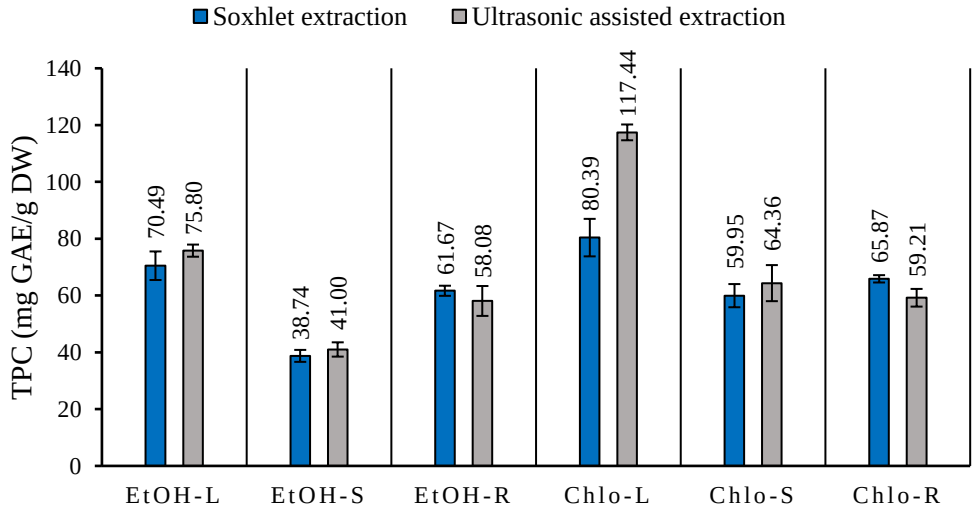

Figure 3. TPC of *C. alata* leaf, stem, and root using SE and UAE

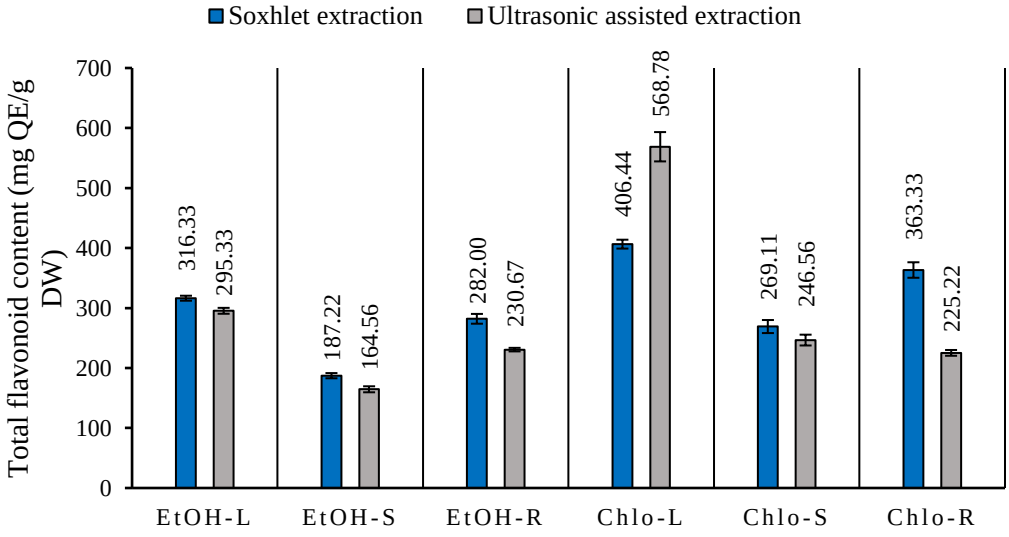

Figure 4. TFC of *C. alata* leaf, stem, and root using SE and UAE

Phytochemical compounds found in different plant parts may be responsible for the unique pharmacological effects in those parts (Sembiring *et al*., 2018). As a common plant response to these stresses, phenolic and flavonoid compounds are produced; these compounds have the ability to repair damaged tissues, absorb UV light, and repel parasites and pathogens (Clement *et al*., 2021). Less exposure is provided to the stems and roots to these factors. Therefore, evolutionary pressures on *C. alata* stems and roots to generate high concentrations of phenolic and flavonoid compounds are lower than on the leaves (Pachorkar & Patil, 2021). Additionally, environmental stressors such as UV radiation, pests, and pathogens affect the foliage more directly (Nantongo *et al*., 2018). As a result, phenolic and flavonoid compounds are more abundant in the leaves of *C. alata* than in its stems and roots. A study by Halim-Lim *et al.* (2020) also reported that the leaves of *C. alata* from water extract showed higher phenolic and flavonoid content than barks and roots.

As for the extraction techniques, UAE showed a better phenolic recovery in all extracts except for the roots extracted from both ethanol and chloroform (EtOH-R, Chlo-R). The ultrasound treatment successfully disrupted the cell wall of the plants, which also reduced particle size and enhanced solvent penetration into the plant matrix. This gave a higher surface of contact between trapped bioactive chemicals and the solvent, which improved the extraction efficiency (Das *et al.*, 2019). Romes *et al.* (2019) also reported the phenolic content of oil palm leaves from UAE was higher than SE and maceration extraction. Long exposure to high temperatures in SE could induce the degradation of bioactive compounds in the plants (Mohammadpour *et al.*, 2019). Therefore, UAE was effective for the recovery of the phenolic compound due to its short optimal extraction time and less quantity of solvents and samples used.

Meanwhile, for TFC, the results showed that SE provided a better recovery of flavonoid, except for the chloroform extract of leaves part (SE-Chlo-L, 406.444 mg QE/g DW) where the ultrasonic extract was higher (UAE-Chlo-L, 568.778 mg QE/g DW). However, the UAE is still competent because, even though the extraction used a small quantity of sample and solvent, it proved to be effective in extracting flavonoids. This agrees with Das *et al.* (2019), where a similar solid-to-solvent ratio was used for sonication, maceration, and soxhlet extraction, proving that sonication provides a better flavonoid recovery than other techniques. A higher solid-to-solvent ratio caused a higher concentration gradient, which raised the diffusion rate of the chemicals from the sample into the solvent (Elboughdiri, 2018: Jovanović *et al.*, 2021).

In this study, all the extracts obtained from chloroform showed a higher TPC compared to ethanol extracts. Similar results are obtained for TFC. A chloroform extract gives better TFC from all parts of *C. alata*, except for the ultrasonic extract of the roots (UAE-Chlo-R, 225.222 mg QE/g DW). The difference was slightly lower than the ultrasonic extract of roots by ethanol (UAE-EtOH-R, 230.667 mg QE/g DW) (Figure 4). These results are attributed to several factors such as the type of extraction solvent, its polarity index, and the solubility of the target compounds in chloroform as extraction solvent (Nguyen *et al.*, 2022).

According to Alara *et al.* (2021), chloroform might selectively extract certain phenolics that are less polar, which are not as effectively extracted by more polar solvents like ethanol or water. This selectivity of solvent extracts could result in a higher concentration of less polar phenolic compounds in chloroform extract. A study by Nguyen *et al.* (2022) also reported that ethyl acetate, acetone, and chloroform extracts of *Avicennia officinalis* L provided a higher TPC than ethanol, methanol, and dichloromethane extracts. Thus, chloroform in combination with UAE is a suitable method for the extraction of phenolic compounds. Meanwhile, SE is a suitable method for the extraction of flavonoids for all plant parts except for the Chlo-L. Based on the results, it is significant to choose the right solvent for phenolic and flavonoid extraction because the combination of extraction methods and solvent selection might provide a variety of outcomes.

CONCLUSION

Different extraction methods and solvent extracts generally affected the yield, phenolic and flavonoid content in *C. alata.* This study showed that extracts from the leaf of *C. alata*

performed better than the root and stem in terms of their extraction yield, TPC and TFC due to biological factors and environmental effects. With the exception of TFC in Chlo-R, the TPC and TFC in stem and root only show slight differences. Also, chloroform is a good choice of solvent for extracting phenolics and flavonoids due to the selectivity of less polar compounds in *C. alata.* For the extraction method, SE is better at extracting flavonoids except for the Chlo-L. Meanwhile, UAE is more efficient in extracting phenolic compounds except for the EtOH-R and Chlo-R. Thus, these findings provide data for selecting methods that produce optimal yield, phenolic and flavonoid contents in *C. alata*'s species.

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