



Phytochemical Characterization, Curcuminoid Quantification by HPLC, and Biological Activities of Ethanolic and Aqueous Extracts of *Zingiber cassumunar* and *Curcuma longa* Prepared by Ultrasonic-Assisted Extraction

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Abstract: *Zingiber cassumunar* and *Curcuma longa* are widely used medicinal plants in Southeast Asia, with therapeutic properties attributed to curcuminoids, phenolics, and flavonoids. This study evaluated the effects of solvent type and ultrasonic pretreatment on extraction efficiency and phytochemical composition. Herbal powders were extracted using water and ethanol under ultrasonic conditions. Extraction yields were determined, and phytochemical contents were analyzed using HPLC for curcuminoids and UV-VIS spectrophotometry for total phenolic content (TPC) and total flavonoid content (TFC). The results demonstrated that extraction efficiency was significantly influenced by solvent selection. Ethanolic extraction produced higher yields and significantly greater concentrations of curcuminoids, phenolics, and flavonoids compared to aqueous extraction. Curcuminoids were predominantly detected in ethanolic extracts, with *Curcuma longa* showing the highest content (13.951% w/w). Similarly, TPC and TFC were markedly higher in ethanolic extracts (412,105.51 mg GAE/kg and 659,001.92 mg QE/kg, respectively), whereas aqueous extracts showed limited recovery due to poor solubility of hydrophobic and semi-polar compounds in water. The enhanced recovery observed with ethanol was attributed to improved solvent penetration and solubilization capacity, further enhanced by ultrasonic cavitation, which promoted cell wall disruption and mass transfer. Additionally, both aqueous and ethanolic extracts of *Z. cassumunar* and *C. longa* demonstrated significant anti-inflammatory activity (inhibition of nitric oxide production in LPS-stimulated macrophages) and antioxidant activity (reduction of intracellular ROS), regardless of solvent type. Overall, the combination of ethanol and ultrasonic pretreatment was identified as a more efficient, less solvent-intensive method for maximizing phytochemical extraction than conventional maceration, supporting its application in functional foods, nutraceuticals, and herbal pharmaceuticals.

Keywords: *Zingiber cassumunar*, *Curcuma longa*, ultrasonic extraction, phytochemical analysis, HPLC

1. Introduction

The *Zingiberaceae* family (*Zingiber cassumunar* and *Curcuma longa*), which includes several economically and pharmacologically significant species,

has been used in traditional medicine, nutraceutical products, and culinary practices in many parts of the world [1]. The quality and pharmacological standardization of these plants, including cassumunar ginger, have been progressively established [2, 3, 4]. It has been determined that the rhizomes of plants in this family are abundant in a variety of bioactive compounds with substantial medicinal potential [5]. Because of its high concentration of curcuminoids, specifically curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which give it its distinctive yellow pigmentation and a variety of biological activities, *C. longa* has drawn a lot of scientific attention among these species [6, 7]. Numerous studies have demonstrated the potent antioxidant, anti-inflammatory, antimicrobial, and anticancer properties of these extracts [8, 9]. Furthermore, phenolic compounds, flavonoids, and terpenoids have been identified in other members of the Zingiberaceae family, further enhancing their therapeutic versatility and health-promoting properties [10, 11]. The development of functional foods, nutraceuticals, and contemporary pharmaceutical products has therefore been seen to depend on the isolation, characterization, and quantification of these phytochemicals.

Because extraction conditions directly affect the yield, purity, and stability of target bioactive compounds, the extraction method used significantly influences the effectiveness and quality of herbal formulations [12]. Long extraction times, high solvent consumption, and possible degradation of thermolabile compounds have been linked to traditional extraction methods such as maceration and percolation [12]. As a result, one of the main goals of current phytochemical research is to develop effective, rapid, and environmentally friendly extraction technologies. In this context, a variety of polar and semi-polar compounds, such as glycosides and curcuminoids, have been proposed for extraction from complex plant matrices using a combination of aqueous and methanolic solvent systems [1]. Ultrasonic-assisted extraction (UAE) has been recognized as a green, innovative technique for phytochemical recovery. This method relies on the generation of acoustic cavitation by applying high-frequency sound waves, leading to the formation and collapse of microbubbles in the solvent medium [13]. The resulting localized high pressure and micro-jets facilitate the disruption of plant cell walls, enhance solvent penetration, and promote the release of intracellular metabolites. As a result, the UAE has been shown to improve extraction efficiency, reduce solvent and energy consumption, and significantly shorten extraction time when compared with conventional extraction methods [13, 14, 15, 16]. Furthermore, because the UAE is a non-thermal, energy-efficient process, the structural integrity and biological activity of thermolabile phytochemicals can be better preserved [17].

In this study, aqueous and ethanolic extracts of Zingiber cassumunar and Curcuma longa prepared using ultrasonic pretreatment were analyzed for phytochemicals. The concentrations and profiles of bioactive constituents were evaluated. An optimized, efficient, and scalable extraction protocol was established. The result of this study is expected to provide an extraction method, the phytochemical composition of these medicinal plants, and to support their potential industrial application in the development of high-value functional and therapeutic products. However, despite the growing literature on individual species, a direct comparison of UAE efficiency using water versus ethanol for both *Z. cassumunar* and *C. longa* simultaneously, combined with integrated curcuminoid HPLC quantification and cellular biological activity assessment, has not been comprehensively reported. This study addresses that gap by evaluating the combined effect of solvent polarity and ultrasonic pretreatment on phytochemical yield, curcuminoid content, TPC, TFC, and biological activities of both species in a single systematic investigation.

2. Materials and Methods

2.1 Herbal Extraction Process

The dried herbs of *Z. cassumunar* and *C. longa* were used in this study and purchased from Fa Sen Herbal Shop (Hatyai, Songkla, Thailand). They were ground into a fine powder and stored in a desiccator until further extraction. To ensure environmental safety, water and 95% (v/v) ethanol were selected as solvents due to their differing charge properties, and the extraction employed a combination of solvent extraction and ultrasonic pretreatment to maximize efficiency. Each herbal sample was extracted at a solid-to-solvent ratio of 1:10, with 10 g of powdered plant material weighed and mixed with 100 mL of water or ethanol. The mixture was agitated using a magnetic stirrer and subsequently incubated for two hours (as a pre-soaking maceration step to promote initial diffusion of soluble compounds before sonication) to facilitate the complete diffusion of bioactive

compounds into the solvent [12]. The mixture was then subjected to ultrasonic pretreatment using an ultrasonic bath operating at 40 kHz and a nominal power output of 150 W (continuous duty cycle) for 60 minutes at a temperature of 40 °C, with the application of ultrasound intended to promote acoustic cavitation and thereby improve mass transfer efficiency within the solvent system [13]. Following pretreatment, the crude extract was separated from the solid residue by filtration, after which the solvent was removed using a rotary evaporator under reduced pressure (approximately 40–50 mbar) at a temperature below 40 °C to prevent thermal degradation of sensitive compounds. The dried extract was used to calculate the extraction yield (%) using equation (1). The extract was stored in a freezer until further use. Finally, the optimized extraction conditions were scaled up by doubling the amounts of both the herbal materials and the solvents to obtain sufficient extract for subsequent characterization.

$$(\text{wt.}\%) = \frac{m_{\text{extract}}}{m_{\text{raw material}}} \times 100 \quad (1)$$

A limitation of this study lies in the potential influence of seasonal variation on the phytochemical composition of the plant materials. The raw materials used were collected from a specific location and at a particular time, which may affect the concentration and profile of bioactive compounds. Variations in environmental factors, such as climate, soil conditions, and the harvesting season, are known to influence the biosynthesis and accumulation of phytochemicals. Therefore, the extraction yields and phytochemical contents reported in this study may not fully represent samples from different regions or harvest periods.

2.2 Characterization of Herbal Extracts

The extracts were evaluated for their sensory and physical characteristics, including appearance, color, odor, clarity, consistency, and phase separation. A series of curcumin standard solutions at concentrations of 0.1, 0.5, 2, 4, 6, 8, 10, and 12 µg/mL was prepared and analyzed by HPLC, with linearity evaluated using the coefficient of determination (R^2) and the relationship between peak area and curcumin concentration established from the calibration curve. Chromatographic analysis was performed using a Shimadzu Nexera LC-40Dx3 Series HPLC system equipped with an autosampler and a photodiode-array (PDA) detector. Separation was achieved on a Zorbax C18 column (150 mm × 4.60 mm, 5 µm particle size), and the mobile phase consisted of 0.1% (v/v) formic acid in water and acetonitrile at a ratio of 50:50 (v/v). Isocratic elution was maintained at 1.0 mL/min, the column temperature was set to 40 °C, and an injection volume of 10 µL was used for all analyses. Detection of curcumin was carried out at a wavelength of 420 nm. The peak was identified by comparing retention times with those of the curcumin standard (Figure 2), and the contents in the extracts were quantified based on peak area.

2.3 Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method, as previously described [25]. The phenolic content of the samples was quantified based on a gallic acid standard calibration curve ($n = 3$), and the results were expressed as milligrams of gallic acid equivalents per kilogram of dry extract (mg GAE/kg).

2.4 Total Flavonoid Content (TFC)

The identification and quantification of flavonoids were performed according to the method described by Lin *et al.* [11]. Detection was performed at 364 nm using a UV–visible detector. Quercetin and apigenin were employed as reference standards for the identification of flavonoid compounds in the samples. Briefly, 0.1 mL of each sample was mixed with 0.3 mL of 5% (w/v) AlCl_3 in methanol and 0.3 mL of 1 M potassium acetate, and the mixture was incubated at room temperature for 30 minutes. Absorbance was measured at 415 nm. Results were expressed as milligrams of quercetin equivalents per kilogram of dry extract (mg QE/kg, $n = 3$).

3. Results and Discussion

3.1 Extracts yield of *Zingiber cassumunar* and *Curcuma longa* from water and ethanol combined with ultrasonic pretreatment

The extraction yields of *Z. cassumunar* and *C. longa* were evaluated using water and ethanol as extraction solvents. There was no significant difference in the percentage yield when the extraction scale was doubled (data not shown). The extraction yields of *Z. cassumunar* using water and ethanol as solvents were 13.07% and 8.77%, respectively. In contrast, the extraction yields of *C. longa* using water and ethanol as solvents were 9.63% and 9.90%, respectively (Figure 1). The result demonstrated that comparative extraction of *Z. cassumunar* performance between the two solvents showed differences in efficiency, depending on solvent polarity and the chemical-specific properties of the target phytochemicals. When water was used as the extraction solvent with *C. longa*, a lower yield was obtained. This finding is consistent with the documented poor aqueous solubility of curcuminoids, which restricts their extraction efficiency in water-based systems [6]. In contrast, ethanolic extraction yielded substantially higher curcumin, ranging from approximately 8.0% to 12.0%. The improved recovery observed with ethanol has been attributed to its ability to dissolve curcuminoids effectively and facilitate deeper solvent penetration into the plant matrix, thereby enhancing mass transfer and extraction efficiency [8, 9]. These results align with previous reports indicating that alcoholic solvents are more suitable for curcumin extraction than aqueous solvents [8, 9]. A similar extraction trend was observed for *Z. cassumunar*. Aqueous extraction, including hydrodistillation or soaking methods, yielded a relatively higher extraction yield of 13.07% by total mass; however, this yield is expected to be largely composed of water-soluble components (polysaccharides, proteins, and other hydrophilic compounds) rather than the non-polar bioactive phenylbutanoids that confer medicinal properties. This limitation has been reported in earlier studies, where water-based extraction methods were found to be ineffective for selectively recovering non-volatile phenylbutanoids such as DMPBD [11]. In contrast, ethanol use significantly increased the total extractable yield to approximately 6–8%. This improvement was attributed to the efficient recovery of non-volatile bioactive compounds, particularly (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD), which has limited water solubility but is readily extracted with organic solvents [11]. These findings further suggest that ethanol is more suitable than water for extracting a broader spectrum of bioactive constituents from *Z. cassumunar*. A significant variation in extraction efficiency was observed between aqueous and ethanolic solvents. Ethanol was shown to be a more effective solvent for plant materials than water. This outcome has been attributed to the physicochemical properties of the major bioactive constituents present in *Z. cassumunar* and *C. longa*, specifically curcuminoids and phenylbutanoids, which exhibit limited solubility in water but higher affinity for polar organic solvents [1, 6]. Previous studies have similarly reported that ethanol enhances the extraction of hydrophobic and semi-polar compounds by penetrating plant cell walls and dissolving a wider range of secondary metabolites [17]. Overall, the results indicate that ethanol is a more effective solvent than water for extracting bioactive compounds from both *Z. cassumunar* and *C. longa*, supporting its use in developing phytochemical-rich extracts for functional and therapeutic applications.

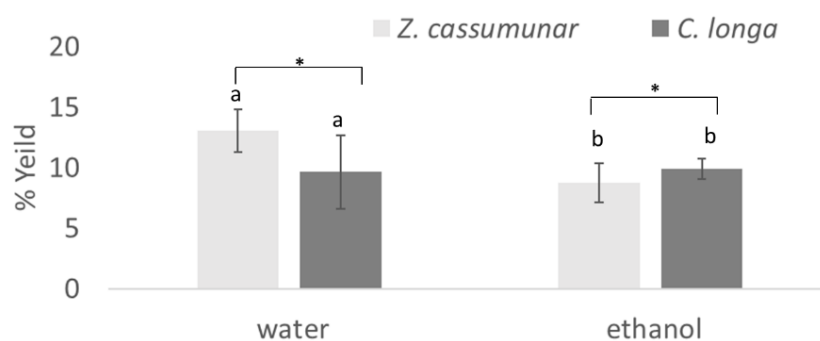


Figure 1. Extraction Yield of *Z. cassumunar* and *C. longa* using water and ethanol as extraction solvents combined with ultrasonic pretreatment. Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by a post hoc test for mean comparison (n=3). Different superscript

letters indicate statistical differences in extraction efficiency between solvents ($p < 0.05$), while asterisks indicate statistical differences between the plant species ($p < 0.05$).

The stability of herbal compress extracts, both in individual and formulaic forms, was evaluated at 4 °C for 90 days. The physical appearance and color of the extracts remained unchanged throughout the study period (data not shown). These findings are consistent with previous reports suggesting that low-temperature storage effectively preserves the organoleptic properties and chemical integrity of bioactive compounds in *Zingiberaceae* plants [18]. Regarding solubility, the extracts of *Z. cassumunar* and *C. longa* obtained via aqueous extraction were found to be highly soluble in water. Conversely, the ethanolic extracts exhibited limited water solubility but were highly soluble in ethanol. This behavior is attributed to the presence of non-polar curcuminoids and essential oil components, which require organic solvents for optimal dissolution [19]. Furthermore, all extracts obtained exhibited a viscous, fine-textured consistency that adhered to the container walls. It was also observed that no phase separation occurred upon prolonged standing, indicating a high degree of physical homogeneity and structural stability within the extract matrix. The concentration of curcumin in the extracts was quantified by High-Performance Liquid Chromatography (HPLC), with results expressed as a percentage by weight (%w/w).

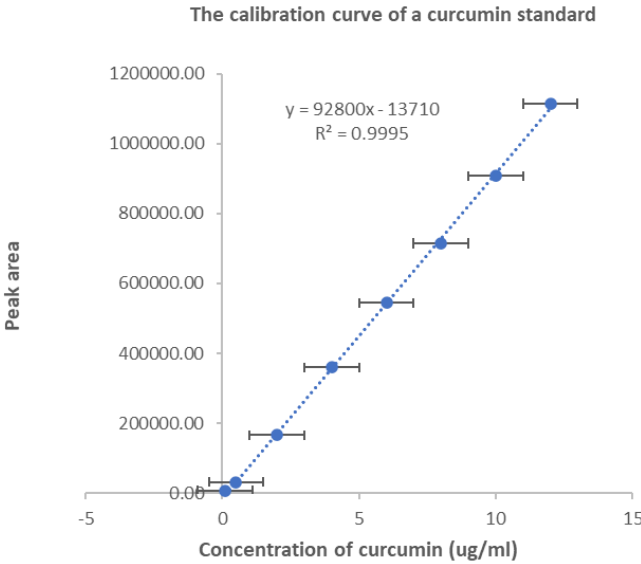


Figure 2. A calibration curve for curcumin at concentrations of 0.1, 0.5, 2, 4, 6, 8, 10, and 12 µg/mL was generated to provide a range of standard concentrations for quantification. (t-test analysis ± SD, n = 3)

A significant disparity in curcumin recovery was observed between the aqueous and ethanolic extraction methods. For the extracts of *Z. cassumunar* (PH₂O) and *C. longa* (CH₂O) prepared using water, the curcumin content was determined to be below the Limit of Detection (< LOD). These observations are consistent with the existing literature, which states that curcumin has extremely low solubility in aqueous media, severely limiting its recovery when water is used as the primary solvent [19].

Table 1. Curcumin Content in *Z. cassumunar* and *C. longa*

Sample	Curcumin (ug/ml±SD)	Curcumin (%w/w±SD)
<i>Z. cassumunar</i> - Water	ND	<LOD ^A
<i>C. longa</i> - Water	ND	<LOD ^A
<i>Z. Cassumunar</i> - Ethanol	0.87 ± 0.03	0.659 ± 0.016
<i>C. longa</i> - Ethanol	6.18 ± 0.37	13.951 ± 0.478

Note: ND= not detection, LOD=0.05µg/mL.

Conversely, the use of ethanol as a solvent facilitated a substantial increase in the extraction of these bioactive compounds. The highest curcuminoid concentration was recorded in the ethanolic *C. longa* extract at

13.951 ± 0.478 %w/w. The ethanolic *Z. cassumunar* extract exhibited a comparatively lower concentration of 0.659 ± 0.016 %w/w (Table 1). Correlated to the phytochemicals analysis of *Z. cassumunar*, which found a phenylbutenoid as a major compound, while the yield of curcumin was 0.2% by weight [20]. These results confirm that organic polar solvents, such as ethanol, are required to effectively penetrate the plant matrix and solubilize hydrophobic polyphenols, such as curcumin [18]. Consistent with previous research on the optimization of component extraction from *Curcuma xanthorrhiza* using Ultrasound-Assisted Extraction (UAE), a high curcumin concentration (39.73% w/w) was obtained [21]. The HPLC analysis also revealed that the ethanolic extract of *C. longa* contained three principal curcuminoids, namely bisdemethoxycurcumin, demethoxycurcumin, and curcumin, with curcumin detected as the dominant compound based on peak area and intensity. The peak area of the ethanolic extract showed a higher peak and greater intensity than those of the aqueous extract (Figures 2 and 3).

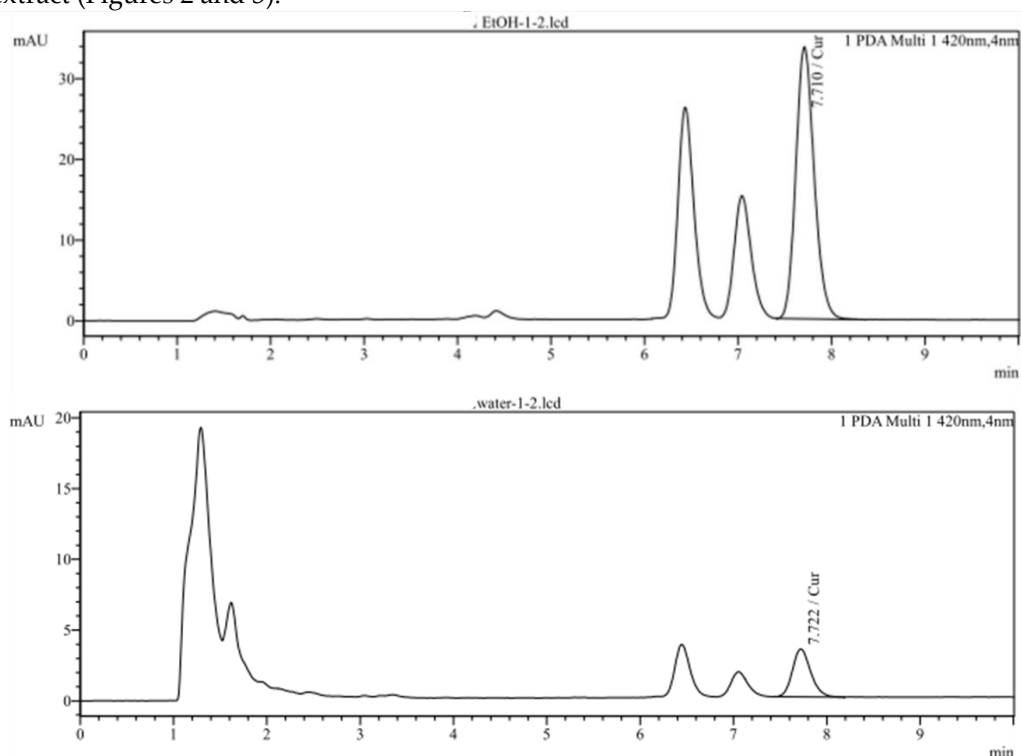


Figure 3. Representative HPLC chromatograms of *C. longa* and *Z. cassumunar* plant extracts showing resolved curcuminoid peaks (bisdemethoxycurcumin, demethoxycurcumin, and curcumin annotated with respective retention times): (top) extract obtained using ethanol as the solvent, and (bottom) extract obtained using water as the solvent.

3.2 Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the herbal extracts was determined using the Folin-Ciocalteu colorimetric method and measured with a UV–VIS spectrophotometer (JASCO V-770). The results were expressed as milligrams of gallic acid equivalents per kilogram of extract (mg GAE/kg $\pm$ SD). The quantitative outcomes are summarized in Table 2 and described below. A substantial difference in the recovery of phenolic compounds was observed between the two extraction solvents. For all samples, TPC values were significantly higher when ethanol was used as the extraction medium than when water was used. The highest phenolic concentration was detected in the ethanolic extract of *C. longa*, with a value of $412,105.51 \pm 23,450.00$ mg GAE/kg. This observation is consistent with previous findings reporting that polar organic solvents, particularly ethanol, exhibit superior efficiency in extracting phenolic compounds from plant matrices when compared with aqueous systems [18]. For *Z. cassumunar*, the phenolic content of the ethanolic extract was determined to be $104,457.14 \pm 7,379.86$ mg GAE/kg, which was approximately fourfold higher than that obtained from the aqueous extract ($28,159.69 \pm 2,215.06$ mg GAE/kg). In the case of *C. longa*, the most pronounced difference between solvents was observed. The ethanolic extract exhibited a TPC value of $412,105.51 \pm 23,450.00$ mg GAE/kg, whereas the

aqueous extract yielded only $17,602.16 \pm 584.06$ mg GAE/kg. The elevated phenolic contents detected in the ethanolic extracts were attributed to the enhanced solubilization of hydrophobic and semi-polar compounds, including curcuminoids and phenylbutanoids, which are known to possess strong antioxidant activity [19]. These findings further confirm the suitability of ethanol as an effective solvent for maximizing the recovery of phenolic constituents from medicinal plant materials.

Table 2. Total Phenolic Content of Herbal Extracts Measured via UV-VIS Spectrophotometry (n=3).

Sample Source	Solvent	Total Phenolic Content (mgGAE/kg $\pm$ SD)
<i>Z. cassumunar</i>	Water (H ₂ O)	$28,159.69 \pm 2,215.06$
<i>C. longa</i>	Water (H ₂ O)	$17,602.16 \pm 584.06$
<i>Z. cassumunar</i>	Ethanol	$104,457.14 \pm 7,379.86$
<i>C. longa</i>	Ethanol	$412,105.51 \pm 23,450.00$

The total flavonoid content (TFC) of the extracts was quantified using UV–VIS spectrophotometry with a JASCO V-770 system. The results were expressed as milligrams of quercetin equivalents per kilogram of extract (mg QE/kg $\pm$ SD) and are presented in Table 3.

Table 3. Total Flavonoid Content of Herbal Extracts Measured via UV-VIS Spectrophotometry (n=3).

Sample Source	Solvent	Total Flavonoid Content (mgQE/kg $\pm$ SD)
<i>Z. cassumunar</i>	Water (H ₂ O)	$8,863.20 \pm 27.15$
<i>C. longa</i>	Water (H ₂ O)	$9,383.57 \pm 22.41$
<i>Z. cassumunar</i>	Ethanol	$70,111.68 \pm 2,831.50$
<i>C. longa</i>	Ethanol	$659,001.92 \pm 7,206.92$

A marked variation in flavonoid recovery was observed depending on the solvent employed for extraction. Across all samples, TFC values were significantly higher in ethanolic extracts than in aqueous extracts. The highest flavonoid concentration was detected in the ethanolic extract of *C. longa*, with a value of $659,001.92 \pm 7,206.92$ mg QE/kg. This outcome is consistent with previous reports showing that polar organic solvents, such as ethanol, enhance the solubility and extraction efficiency of polyphenolic compounds, including flavonoids, through favorable solvent–solute interactions [22]. For *Z. cassumunar*, the TFC of the ethanolic extract was determined to be $70,111.68 \pm 2,831.50$ mg QE/kg, approximately eightfold higher than that of the aqueous extract ($8,863.20 \pm 27.15$ mg QE/kg). In the case of *C. longa*, the ethanolic extract exhibited a markedly higher flavonoid concentration ($659,001.92 \pm 7,206.92$ mg QE/kg) than the aqueous extract, which contained only $9,383.57 \pm 22.41$ mg QE/kg. The superior recovery of flavonoids in ethanolic extracts was attributed to the enhanced ability of ethanol to penetrate plant cell walls and solubilize semi-polar and non-polar bioactive compounds. Additionally, ethanol facilitates disruption of hydrogen bonds between flavonoids and cell wall polysaccharides, thereby promoting their release into the extraction medium [23].

3.3 Comparative Analysis of Total Phenolic and Flavonoid Contents

A comparative evaluation of total phenolic content (TPC) and total flavonoid content (TFC) in *Z. cassumunar* and *C. longa* was conducted to assess the influence of solvent selection on the recovery of bioactive compounds. The results are summarized below and illustrated in the corresponding graphical representation. A pronounced disparity in phenolic and flavonoid concentrations was observed between aqueous and ethanolic extraction methods. For both plant species, significantly higher levels of bioactive compounds were obtained with ethanol than with water. In *C. longa*, the ethanolic extract exhibited a total phenolic content of $412,105.51 \pm 23,450.00$ mg GAE/kg and a total flavonoid content of $659,001.92 \pm 7,206.92$ mg QE/kg. In contrast, the aqueous extract yielded substantially lower concentrations: $17,602.16 \pm 584.06$ mg GAE/kg for TPC and $9,383.57 \pm 22.41$ mg QE/kg for TFC. A similar extraction pattern was observed for *Z. cassumunar*. The ethanolic extract produced higher concentrations of phenolics and flavonoids, with TPC and TFC values of $104,457.14 \pm 7,379.86$ mg GAE/kg and $70,111.68 \pm 2,831.50$ mg QE/kg, respectively. Conversely, the aqueous extract yielded markedly lower values of $28,159.69 \pm 2,215.06$ mg GAE/kg for TPC and $8,863.20 \pm 27.15$ mg QE/kg for TFC.

The relationship between phenolic and flavonoid contents was found to be strongly influenced by both plant species and extraction solvent. In aqueous extracts, TPC values were generally higher than TFC values for *Z. cassumunar*, whereas *C. longa* exhibited relatively balanced but overall low concentrations of both compound classes (Figure 4). In contrast, ethanolic extracts of *C. longa* demonstrated a markedly higher flavonoid content compared to phenolic content, with TFC values exceeding TPC by more than 50%. This observation is consistent with the high solubility of curcuminoids, which are flavonoid-related polyphenols, in polar organic solvents such as ethanol. Although flavonoids are a subclass of phenolic compounds, the higher TFC values compared to TPC may be attributed to differences in assay principles and calibration standards (quercetin vs. gallic acid equivalents), as well as the strong complex-forming capacity of flavonoids with $AlCl_3$ [24, 25]. Based on the comparative results, ethanol was identified as the superior solvent for the efficient recovery of both phenolic and flavonoid compounds from *Z. cassumunar* and *C. longa* rhizomes. The predominance of hydrophobic and semi-polar bioactive constituents in these species necessitates the use of a polar organic solvent to maximize extraction efficiency and enhance the potential therapeutic value of the resulting extracts.

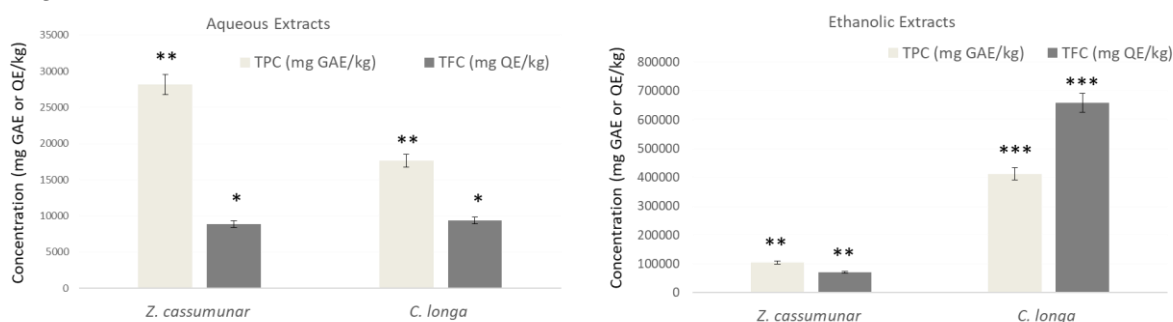


Figure 4. Comparison of total Phenolic and Flavonoid content in *Zingiber cassumunar* and *Curcuma longa*. For comparison of the mean values, one-way ANOVA followed by post hoc was used ($p < 0.05$, $n = 3$). Asterisks indicate statistical differences in TPC and TFC content between different plants (* no differences in TFC content, ** and *** differences in TPC and TFC content)

3.4 Biological activities of *Zingiber cassumunar* and *Curcuma longa* extracts

The cytotoxic effects of *Z. cassumunar* and *C. longa* extracts were evaluated using the MTT assay following 24-hour incubation with macrophage cells at concentrations of 0.25, 0.5, 1, 2, 5, and 10 $\mu\text{g/mL}$. The results indicated that aqueous extracts from both plant species did not induce cytotoxicity, as cell viability remained comparable to that of the control group (data not shown). In contrast, the ethanolic extract of *C. longa* exhibited concentration-dependent cytotoxicity, with cell viability remaining above 85% at concentrations ranging from 0.25 to 5 $\mu\text{g/mL}$, while a significant reduction in cell viability was observed at 10 $\mu\text{g/mL}$. Meanwhile, the ethanolic extract of *Z. cassumunar* did not exhibit cytotoxicity at any tested concentration (Figure 5).

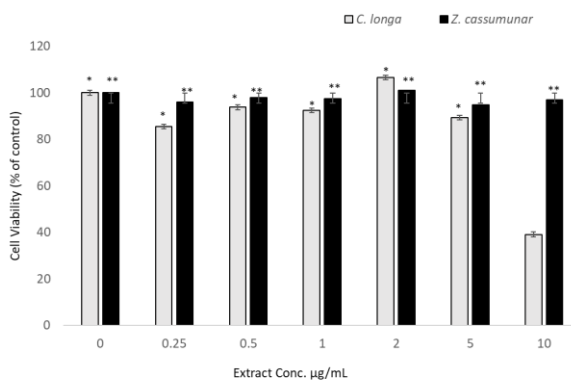


Figure 5. Cell viability of macrophage cells treated with *Z. cassumunar* and *C. longa* extracts at concentrations of 0.25, 0.5, 1, 2, 5, and 10 $\mu\text{g/mL}$. For comparison of the mean values, one-way ANOVA followed by post hoc tests was used ($p < 0.05$; $n = 3$). *Cell viability with 85% of *C. longa* extracts ** no difference of cell viability

Nitric oxide production in LPS-stimulated macrophage cells was evaluated following 24-hour treatment with *Z. cassumunar* and *C. longa* extracts. The results demonstrated that both aqueous and ethanolic extracts at 5 µg/mL significantly reduced NO production to levels comparable to those of the untreated control group (without LPS stimulation), without inducing significant cytotoxicity (data not shown). Therefore, a concentration of 5 µg/mL was determined to be the optimal concentration for extracts derived from both herbal sources. Both aqueous and ethanolic extracts at 5 µg/mL significantly reduced nitric oxide levels to levels comparable to those of the non-LPS control group (Fig. 6). Similar inhibitory effects were observed for both plant extracts, and no substantial differences in nitric oxide suppression were detected between water and ethanol extracts at the same concentration. These findings indicate that both *Z. cassumunar* and *C. longa* extracts exhibited anti-inflammatory activity (Figure 6), regardless of the solvent used [26, 27, 28].

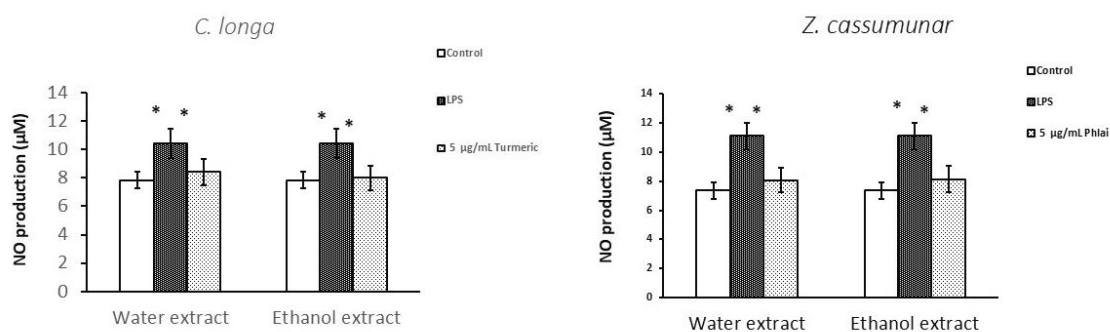


Figure 6. Nitric oxide production in LPS-stimulated macrophage cells was evaluated following 24-hour treatment with *Z. cassumunar* and *C. longa* extracts. For comparison of mean values, a one-way ANOVA followed by post hoc tests was used. ** $p < 0.05$ vs. LPS-treated control ($n = 3$).

The optimal incubation period for antioxidant activity assessment was determined using RAW 264.7 macrophage cells stimulated with LPS and incubated for 1, 3, 6, 12, and 24 hours. Intracellular reactive oxygen species (ROS) levels were measured using a microplate reader. The highest ROS production was observed after 3 hours of incubation (Fig. 7); therefore, this time point was selected as the optimal condition for subsequent antioxidant assays. Under these conditions, the antioxidant activities of *Z. cassumunar* and *C. longa* extracts were evaluated. At a concentration of 5 µg/mL, both aqueous and ethanolic *Z. cassumunar* extracts significantly reduced ROS levels compared with the LPS-treated control group, indicating effective antioxidant activity. Similarly, both aqueous and ethanolic *C. longa* extracts at the same concentration markedly decreased ROS production relative to the LPS control. Overall, antioxidant effects were demonstrated for both *Z. cassumunar* and *C. longa* extracts, regardless of solvent type, as evidenced by a significant reduction in intracellular ROS under LPS-induced oxidative stress conditions [29, 32].

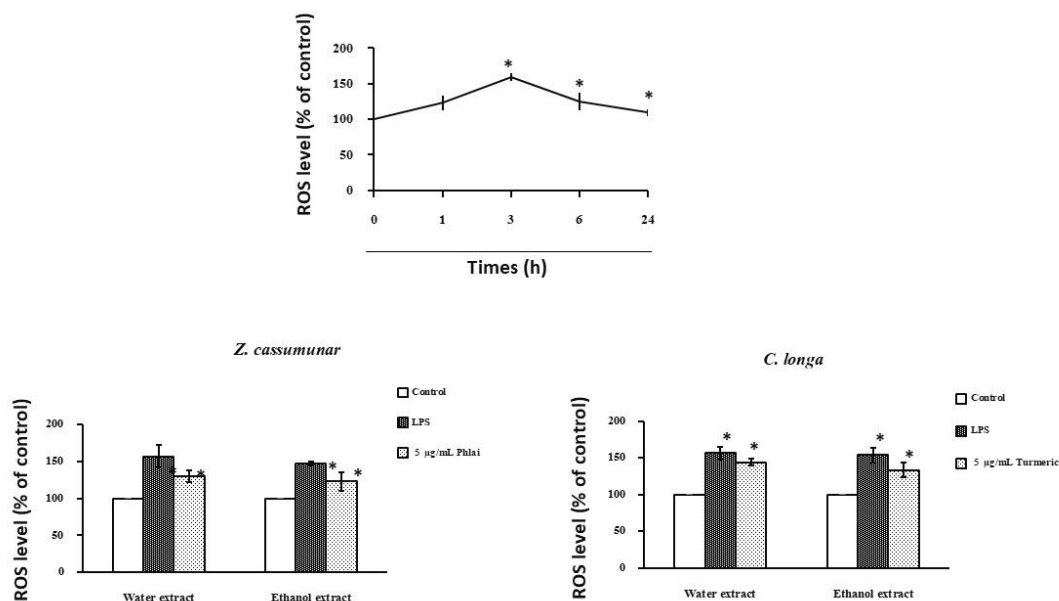


Figure 7. Intracellular reactive oxygen species (ROS) levels of *Zingiber cassumunar* and *Curcuma longa* from aqueous and ethanolic extracts. The y-axis label “ROS level (% of control)” is expressed relative to the untreated control group (= 100%). LPS-stimulated cells exceeding 100% at 3 h indicate elevated oxidative stress above baseline, consistent with the expected positive-control response. Authors should clarify this in the legend text to avoid confusion. Statistical significance indicators * $p < 0.05$ vs. LPS-treated group (one-way ANOVA with Tukey’s post-hoc test; $n = 3$).

4. Conclusion

This study demonstrated that solvent selection significantly influences the extraction efficiency of bioactive compounds from *Z. cassumunar* and *C. longa*. Ethanol proved to be superior to water, yielding higher concentrations of curcuminoids, phenolics, and flavonoids, as confirmed by HPLC, TPC, and TFC analyses. Enhanced recovery is attributed to ethanol’s ability to solubilize semi-polar and hydrophobic phytochemicals. Furthermore, ultrasonic pretreatment combined with ethanolic extraction improved overall extraction efficiency while maintaining phytochemical integrity. These findings support the use of ethanol as an optimal solvent and provide a scientific basis for developing standardized extraction protocols for functional foods, nutraceuticals, and herbal pharmaceuticals. Collectively, the results demonstrated that ethanol is the most suitable solvent for the extraction of phenolics, flavonoids, and curcuminoids from *Z. cassumunar* and *C. longa*. The combined use of ultrasonic pretreatment and ethanolic extraction was shown to enhance extraction efficiency while preserving phytochemical integrity. These findings provide a scientifically validated basis for optimizing extraction protocols and support the potential industrial application of these medicinal plants in the development of functional foods, nutraceuticals, and herbal pharmaceutical products [30, 31]. Nevertheless, several limitations of the present study should be acknowledged. Biological activity was assessed at a single concentration (5 µg/mL) only, precluding the calculation of IC_{50} values and dose-response relationships. Cytotoxicity of the extracts toward RAW 264.7 cells was not evaluated, and confirmation that the observed reduction in NO and ROS reflects true pharmacological activity rather than cell death requires further investigation. Additionally, plant materials were sourced commercially without voucher specimen deposition, which limits batch-to-batch reproducibility. Future studies should employ multi-concentration biological assays with full IC_{50} determination, conduct in vivo validation, include cytotoxicity controls, use authenticated herbarium-vouchered plant material, and explore encapsulation or formulation strategies to enhance the bioavailability of the predominantly hydrophobic bioactive compounds identified.

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References

- [1] Sumi, M. J.; Zaman, S. B.; Imran, S.; Sarker, P.; Rhaman, M. S. A Review on the Ethnopharmacological Importance and Biochemical Composition of Medicinal Plants within the Zingiberaceae Family. *Plant Sci. Today* **2024**, 11(sp1). <https://doi.org/10.14719/pst.3514>
- [2] Srisuwan, T.; Keereekoch, T.; Chikasem, I.; Da-o, A.; Salaemae, M.; Saleh, A.; Subhadhirasakul, S. Development of the Preparation Methods for Qualified Cassumunar Ginger Powder Based on Thai Herbal Pharmacopoeia Standards. *Thaksin Univ. J.* **2016**, 19(2) 53–65.
- [3] Khamsopa, N.; Vanichanon, A.; Sukrakanchana, N. Efficacy of Essential Oils from Clove (*Syzygium aromaticum*) and Cassumunar Ginger (*Zingiber montanum*) as Anesthetic Agents for Fruit Fly (*Drosophila melanogaster*). *ASEAN J. Sci. Technol. Rep.* **2018**, 21(3), 58–64.
- [4] Pascual, P. R. L.; Remedios, E. A.; Abello, N. F.; Carabio, D. E.; Pascual, V. U.; Alburo, R. P. Vegetative, Photosynthetic, and Anthocyanin Content of Turmeric (*Curcuma longa* L.) Applied with Organic Foliar Fertilizers. *ASEAN J. Sci. Technol. Rep.* **2025**, 28(5), e257477. <https://doi.org/10.55164/ajstr.v28i5.257477>
- [5] Ali, A. M. A.; El-Nour, M. E. M.; Yagi, S. M. Total Phenolic and Flavonoid Contents and Antioxidant Activity of Ginger (*Zingiber officinale* Rosc.) Rhizome, Callus and Callus Treated with Some Elicitors. *J. Genet. Eng. Biotechnol.* **2018**, 16(2), 677–682. <https://doi.org/10.1016/j.jgeb.2018.03.003>
- [6] Aggarwal, B. B.; Harikumar, K. B. Potential Therapeutic Effects of Curcumin, the Anti-Inflammatory Agent, against Neurodegenerative, Cardiovascular, Pulmonary, Metabolic, Autoimmune and Neoplastic Diseases. *Int. J. Biochem. Cell Biol.* **2009**, 41(1), 40–59. <https://doi.org/10.1016/j.biocel.2008.06.010>
- [7] Keereekoch, T.; Srisuwan, T.; Taleh, R.; Hemtragoonwong, R.; Chesa-E, S.; Subhadhirasakul, S. Effects of Steam Sterilization Time and Temperature on Quality of Turmeric Powder. *Thaksin Univ. J.* **2014**, 17(1), 57–67.
- [8] Akbik, D.; Ghadiri, M.; Chrzanowski, W.; Rohanizadeh, R. Curcumin as a Wound Healing Agent. *Life Sci.* **2014**, 116(1), 1–7. <https://doi.org/10.1016/j.lfs.2014.08.016>
- [9] Kocaadam, B.; Şanlıer, N. Curcumin, an Active Component of Turmeric (*Curcuma longa*), and Its Effects on Health. *Crit. Rev. Food Sci. Nutr.* **2017**, 57(13), 2889–2895. <https://doi.org/10.1080/10408398.2015.1077195>
- [10] Rawat, G.; Geetha, S. J.; Pandit, S.; et al. Flavonoids as Antioxidants and Anti-Inflammatory Agents in Cancer Prevention. *Vegetos* **2026**. <https://doi.org/10.1007/s42535-026-01670-2>
- [11] Lin, L. Z.; Mukhopadhyay, S.; Robbins, R. J.; Harnly, J. M. Identification and Quantification of Flavonoids of Mexican Oregano (*Lippia graveolens*) by LC-DAD-ESI/MS Analysis. *J. Food Compos. Anal.* **2007**, 20(5), 361–369. <https://doi.org/10.1016/j.jfca.2006.09.005>
- [12] Pauner, R.; Hamzah, A. S. Phenylbutanoid Compounds from *Zingiber cassumunar* and Their Pharmacological Activities. *J. Ethnopharmacol.* **2022**, 289, 115016
- [13] Chemat, F.; Rombaut, N.; Zill-e-Huma; Khan, M. K. Ultrasonic-Assisted Extraction of Food Ingredients. *Ultrason. Sonochem.* **2017**, 34, 540–549. <https://doi.org/10.1016/j.ultsonch.2016.06.035>

- [14] Saenge Chooklin, C.; Chooklin, S. Optimized Extraction of Total Phenolic Compounds from 'Tubtim Siam' Pummelo Peel Using Ultrasonic Technique and Response Surface Methodology. *ASEAN J. Sci. Technol. Rep.* **2021**, 24(1), 61-70. <https://doi.org/10.55164/ajstr.v24i1.240796>
- [15] Buachoon, N. Development of Ultrasonic Extraction Techniques for Bioactive Compounds from Banana Peel Waste: A Comparative Study of Biological Activities. *ASEAN J. Sci. Technol. Rep.* **2025**, 29(1), e258852. <https://doi.org/10.55164/ajstr.v29i1.258852>
- [16] Panyo, J.; Klomklao, S. Microwave-Assisted Extraction of Phenolic Compounds from *Smilax ovalifolia* Roxb. and Chemical Compositions. *Thaksin Univ. J.* **2013**, 45–51. <https://doi.org/10.55164/ajstr.v26i3.249604>
- [17] Sridhar, N.; Veer-Anjaneyulu, A.; Arora, A. Phytochemical Analysis and Biological Activities of Ethanolic Extract of *Curcuma longa* Rhizome. *Braz. J. Biol.* **2021**, 81(3), 737–740. <https://doi.org/10.1590/1519-6984.230628>
- [18] Peram, M. R.; Jalalpure, S. S.; Palkar, M. B.; Diwan, P. V. Stability Studies of Pure and Mixture Form of Curcuminoids by Reverse Phase-HPLC Method under Various Experimental Stress Conditions. *Food Sci. Biotechnol.* **2017**, 26(3), 591–602. <https://doi.org/10.1007/s10068-017-0087-1>
- [19] Revathy, S.; Elumalai, S.; Bennita, M. A.; Prathapa, S. T. Isolation, Purification and Identification of Curcuminoids from *Curcuma longa* L. by Column Chromatography. *J. Exp. Sci.* **2011**, 2(7), 21–25.
- [20] Han, A.-R.; Kim, H.; Piao, D.; Jung, C.-H.; Seo, E. K. Phytochemicals and Bioactivities of *Zingiber cassumunar* Roxb. *Molecules* **2021**, 26(8), 2377. <https://doi.org/10.3390/molecules26082377>
- [21] Azemi, N.; Basar, N.; Toemen, S. Ultrasound-Assisted Extraction (UAE) of Phytochemicals with Response Surface Methodology (RSM) in *Curcuma xanthorrhiza*. *Ann. Trop. Med. Public Health* **2020**, 13(8). <https://doi.org/10.36295/ASRO.2020.231306>
- [22] Rajchasom, S.; Sombatnan, P.; Vuthijumnonk, J. T.; Suphiratwanich, P. Comparison of Different Extraction Methods for Bioactive Compounds from Kao-Kum Doi-Saket (*Oryza sativa* L.). *ASEAN J. Sci. Technol. Rep.*, 38–46. <https://doi.org/10.55164/ajstr.v26i4.249961>
- [23] Ruiz-Vasquez, L.; Ruiz Mesia, L.; Maco, M. M.; Zapata, J. A.; Ricopa Cotrina, H.; Cobos, M.; Pinedo-Cancino, V.; Tello, F.; Castro, J. C. Optimized Folin-Ciocalteu Method for Determination of Total Polyphenols in Medicinal Plants of the Peruvian Amazon: Validation and Application to Twelve Species. *AppliedChem* **2026**, 6(1), 17. <https://doi.org/10.3390/appliedchem6010017>
- [24] Chang, C. C.; Yang, M. H.; Wen, H. M.; Chern, J. C. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *J. Food Drug Anal.* **2002**, 10(3), 178–182. <https://doi.org/10.38212/2224-6614.2748>
- [25] Singleton, V. L.; Orthofer, R.; Lamuela-Raventós, R. M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods Enzymol.* **1999**, 299, 152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- [26] Chaijan, S.; Chaijan, M.; Uawisetwathana, U.; Panya, A.; Phonsatta, N.; Shetty, K.; Panpipat, W. Phenolic and Metabolic Profiles, Antioxidant Activities, Glycemic Control, and Anti-Inflammatory Activity of Three Thai Papaya Cultivar Leaves. *Foods* **2024**, 13(11), 1692. <https://doi.org/10.3390/foods13111692>
- [27] Bunkaew, K.; Ponpichai, P.; Lertworapreecha, M.; Maitip, J.; Chanasit, W. Phytochemical Analysis and Biological Activities of Propolis from *Geniotrigona thoracica*: Evaluating Its Therapeutic Applications. *ASEAN J. Sci. Technol. Rep.* **2024**, 27(5), e253219. <https://doi.org/10.55164/ajstr.v27i5.253219>
- [28] Sueree, L.; Madaka, F.; Jongrungruangchok, S.; Vipunngun, N.; Songsak, T. Comprehensive Pharmacognostic Analysis and Quality Control Parameters of *Cannabis sativa* L. subsp. *sativa*: Evaluating Leaves and Flowers for Medicinal Applications. *ASEAN J. Sci. Technol. Rep.* **2025**, 28(6), e259515. <https://doi.org/10.55164/ajstr.v28i6.259515>
- [29] Altaweel, D. Enhancement of Bioactive Compounds and Nutrient Content in Rosemary (*Salvia rosmarinus*) Using Nano-Magnesium and NPK Fertilization: A GC-MS Analysis. *ASEAN J. Sci. Technol. Rep.* **2025**, 28(6), e261168. <https://doi.org/10.55164/ajstr.v28i6.261168>
- [30] Worawut, K.; Phungpis, B.; Noppawan, P. Valorization of White Shrimp Shell Waste: Development of Chitosan-Based Pellet Feed for Enhanced Nile Tilapia (*Oreochromis niloticus*) Nutrition. *ASEAN J. Sci. Technol. Rep.* **2026**, 29(2), e260098. <https://doi.org/10.55164/ajstr.v29i2.260098>

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- [31] Sang-Ngam, S.; Chaowiset, W.; Theerapisit, S.; Promprao, S.; Sintupachee, S. Ecological Factors Governing the Persistence of Khlong-Saeng Durian: Implications for Conservation. *ASEAN J. Sci. Technol. Rep.* e259593. **2025**, 28(5), <https://doi.org/10.55164/ajstr.v28i5.259593>
- [32] Tongkasee, P.; Srithat, D.; Srithupthai, K.; Wechvitan, P.; Thititanaapipong, P.; Insuwan, W.; Kamsri, P.; Khotpakdee, P. Extraction and Phytochemical Profile of Three Herbal Weeds: *Chromolaena odorata* L., *Amaranthus viridis* L., and *Cyperus rotundus* L. for Green Synthesis of Silver Nanoparticles. *ASEAN J. Sci. Technol. Rep.* **2023**, 26(3), 10–23. <https://doi.org/10.55164/ajstr.v26i3.249267>