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The Effect of Blanching, Extraction Methods, and Solvent on Antioxidant Activity, Total Phenols, Flavonoids, and Tannins in *Zingiber zerumbet L.* Rhizome

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Abstract

Zingiber zerumbet L. has long been used as one of the most popular herbal remedies for treating a wide range of common ailments. This research aimed to evaluate the total levels of tannins, phenols, flavonoids, and antioxidant activity in *Z. zerumbet* rhizome extract, obtained from 9 - 12 month rhizomes, extract after undergoing a blanching process with 0.05 % citric acid, followed by different extraction methods and solvents. Antioxidant and bioactive component tests were analyzed using 2-way ANOVA, while a t-test was employed to compare the effects of ethanol and distilled water as solvents, assessing their statistical significance. Pearson's test was used to analyze correlations between variables. A strong positive correlation was observed between total phenol, tannin, and flavonoid content, while IC_{50} values for DPPH and ABTS assays negatively correlated with bioactive compounds. Blanching *Z. zerumbet* rhizomes with citric acid, combined with ethanol as the solvent, 24-h maceration, and 30-min sonication resulted in optimal antioxidant capacity and bioactive compound extraction. Blanching with 0.05 % citric acid, followed by extraction using ethanol, 24-h maceration, and 30-min sonication, was identified as the most effective method for maximizing the antioxidant potential of *Z. zerumbet*. Blanching was found to affect the morphology and solubility of the powdered samples. The FTIR spectra of the powder and extract samples of *Z. zerumbet* reveal a broad stretching vibration of the O–H bond, along with other detected bonds such as CH_2, C=C, and C–N. The extract sample displays new absorption bands, suggesting the successful extraction of additional compounds during the treatment process. This study underscores the importance of proper extraction methods and pretreatment in maximizing the therapeutic potential of *Z. zerumbet* as a natural antioxidant source.

Keywords: Antioxidative properties, Blanching, Extraction method, Functional group, *Zingiber zerumbet* L. rhizome

Introduction

Zingiber zerumbet L. is widely used in traditional medicine across various cultures, addressing a wide range of health conditions. It is utilized in traditional medical systems such as Ayurveda, Unani, and Chinese medicine to treat digestive disorders, microbial infections, and bleeding issues [1] *Z. zerumbet* contains various compounds like pinene, humulene, linalool, caryophyllene, borneol, limonene, curcuminoids, kaempferol, and zerumbone [2,3]. These bioactive compounds are known for their diverse pharmacological effects, including analgesic, anti-inflammatory, antihyperlipidemic, antineoplastic, immunomodulatory,

antioxidant, antipyretic, hepatoprotective, nephroprotective, and gastroprotective activities [4]. The effectiveness of these active compounds in food ingredients like *Z. zerumbet* can be influenced by processing methods. Several studies have shown that heat treatments, such as blanching, can enhance the active components.

A study conducted by Pujimulyani *et al.* [5] on white turmeric rhizomes showed that blanching in a 0.05 % citric acid solution at 100 °C for 5 min significantly increased antioxidant activity, total phenol content, total flavonoid content, and condensed tannin content compared to unblanched white turmeric [5]. Furthermore, Pujimulyani *et al.* [6] reported that black turmeric powder subjected to blanching exhibited higher antioxidant activity than non-blanched samples [6]. Blanching with 0.05 % citric acid for 5 min was the most effective, yielding the highest levels of phenolics, flavonoids, and tannins. This increase in antioxidant activity is believed to be due to blanching facilitating the release of antioxidant components from the cell structure, thus improving extraction efficiency. The extraction method is also crucial in determining the amount of bioactive components obtained. Choosing the proper extraction method is essential to maximize yields. For instance, a study by Momchev *et al.* [7] found that extracting *Echinacea purpurea* using a water-ethanol or water-glycerol solvent mixture demonstrated significant radical-scavenging activity [7]. Moreover, ultrasonic extraction has been shown to produce higher yields of phenolic acids in a shorter time. Oroian *et al.* [8] also reported that ultrasonic extraction yielded higher total phenol content than microwave extraction and maceration in propolis samples [8].

This study aims to evaluate the potential of *Z. zerumbet* as an ethnomedicine-based food ingredient by investigating the effects of various pretreatment processes and extraction conditions on its bioactive compound content and antioxidant activity. Most previous studies have focused only on the general phytochemical profile and its pharmacological benefits, without considering how pretreatment processes and extraction techniques can affect the bioactive compound content and extraction efficiency. The blanching process was tested with 3 treatments: Non blanching, blanching, and blanching with a 0.05 % citric acid solution. Additionally, various extraction methods and durations were applied, including 24-h maceration, 48-h maceration, 15-min sonication, and 30-min sonication, using ethanol and distilled water as solvents. The impact of these treatments was analyzed through phytochemical testing, including the determination of total phenol, flavonoid, and tannin content, as well as the measurement of antioxidant capacity using DPPH and ABTS assays. Furthermore, morphological and structural changes from powdered samples to extracts were confirmed and characterized using SEM and XRD, while FTIR was used to provide insight into modifications in functional group resulting from the applied treatments. This study aims to determine the optimal pretreatment conditions and extraction methods to maximize the bioactive content and functional properties of Z. zerumbet for applications in the food and pharmaceutical industries.

Materials and methods

Preparation and extraction of *Z. zerumbet*

In this study, the rhizome of *Z. zerumbet* (9 - 12 months) was utilized as the primary material for analysis. The rhizomes were thoroughly cleaned to remove roots, stems, and adhering soil before being sliced into 2 mm thick pieces using a slicing tool. The preparation of the simplicia samples involved 3 blanching treatments: (1) Blanching using only water as the steaming medium (1700 mL), (2) Blanching using a citric acid solution, where 0.05 % citric acid (Cap Gajah) was dissolved in 1700 mL of water as the steaming medium, and (3) A control treatment without blanching. In the blanching process, the rhizome slices were steamed at 100 °C for 10 min. Following the steaming process, the rhizomes were dried in a food dehydrator at 50 °C for 24 h. Once dried, the rhizome samples were ground into a fine powder using a blender, and the resulting powder was sieved through an 80-mesh sieve. For the extraction process, the powder was dissolved in 2 types of solvents (96 % ethanol and distilled water) at a 1:10 ratio of powder to solvent. Two extraction methods were employed: Maceration, with time intervals of 24 and 48 h, and sonication, with durations of 15 and 30 min. Sonication was performed using an ultrasonic cleaner (Ultrasonic Cleaner DELTA D68H) operating at a frequency of 20 kHz. After extraction, the mixture was filtered to remove the solvent, followed by vacuum filtration to separate the filtrate and powder.

The resulting filtrate was evaporated using a rotary evaporator (RV 10 Digital Diagonal IKA) until the final extract was obtained.

Measurement of antioxidant capacity

DPPH radical scavenging assay

The antioxidant activity test was performed using the spectrophotometric method Sari *et al.* [9] by calculating the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) reduction. A total of 1 mL of diluted extract in ethanol was added to 1 mL of DPPH (0.15 mM in ethanol), and at the same time, a control consisting of 1 mL of DPPH with 1 mL of ethanol was prepared. The solution was mixed thoroughly and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm. The determination of antioxidant activity is expressed in IC_{50} (µg/mL) as antioxidant capacity. The IC_{50} value (the concentration of the sample required to inhibit 50 % of DPPH radicals) was determined by plotting the percentage of inhibition against different extract concentrations and calculating the regression equation. The IC_{50} was obtained as the x-value corresponding to 50 % inhibition.

ABTS radical scavenging assay

The antioxidant activity was assessed using the ABTS method (2,2-Azinobis(3-ethylbenzothiazoline)-6-sulfonic acid) following the procedure outlined by Sami and Rahimah with slight modifications [10]. Initially, 2 stock solutions were prepared: (A) containing 7.1015 mg of ABTS and (B) containing 3.500 mg of K_2S_2O8. Both solutions were dissolved in 5 mL of distilled water and incubated for 12 h. After the incubation period, solutions A and B were combined in the dark and diluted with absolute ethanol until the total volume reached 25 mL. To prepare the ABTS blank solution, 1 mL of the ABTS stock solution was mixed with absolute ethanol to a final volume of 5 mL. Subsequently, 100, 200, 300, 400, and 500 µL of the sample solution, with a concentration of 1000 µg/mL, were added to 1 mL of ABTS solution, followed by the addition of absolute ethanol to reach a final volume of 5 mL, resulting in final concentrations of 20, 40, 60, 80, and 100 µg/mL. The absorbance values of the samples and the blank were then measured at a wavelength range of 745 - 755 nm. The determination of antioxidant activity is expressed in IC_{50} (µg/ml) as antioxidant capacity.

Total phenolic

The total phenol content was measured by dissolving approximately 0.3 g of extract in a 10 mL mixture of ethanol and distilled water (1:1 ratio). A 0.2 mL aliquot of the extract was taken and mixed with 15.8 mL of distilled water and 1 mL of 50 % (v/v) Folin-Ciocalteu reagent (Merck, USA). The mixture was left to stand for 8 min, after which 3 mL of 5 % (w/v) Na_2CO_3 (Sigma-Aldrich, USA) was added. The solution was kept in the dark at room temperature (28 ± 3 °C) for 2 h. Absorbance was measured at 725 nm using a BioSpectrometer (Eppendorf, Germany), and the values were compared to a standard curve of gallic acid (Sigma-Aldrich, USA). The total phenol content was expressed in milligrams of gallic acid equivalent per kg of dry weight (mg GAE/kg DW) [11-13]

Total tannin

The total tannin content was determined using the following method: 0.5 g of extract was macerated in 10 mL of diethyl ether (Merck, USA) for 20 h, then filtered. The residue was boiled with 100 mL of distilled water for 2 h and allowed to cool. A 0.1 mL portion of the cooled sample was mixed with 0.1 mL of Folin-Ciocalteu reagent (Merck, USA), vortexed, followed by the addition of 2 mL of 2 % Na_2CO_3 (Sigma-Aldrich, USA), and vortexed again. The absorbance was measured at 760 nm using a BioSpectrometer (Eppendorf, Germany) after incubating the solution at room temperature (28 ± 2 °C) for 30 min. The results were compared against a tannic acid standard curve (Sigma-Aldrich, USA) prepared similarly, and the total tannin content was expressed in milligrams of tannic acid per kg of dry weight (mg TAE/kg DW) [11-13]

Total flavonoid

The total flavonoid content was measured as follows: 1 mg of encapsulated MCV was weighed and dissolved in 10 mL of 95 % ethanol (SmartLab, Indonesia). Then, 0.7 mL of distilled water was added to the ethanol-dissolved encapsulate. Next, 0.1 mL of 5 % $NaNO_2$ (Sigma-Aldrich, USA) was added. After 5 min, 0.1 mL of 10 % $AlCl_3$ (Sigma-Aldrich, USA) was introduced, followed by the addition of 0.5 mL of 1 M

NaOH (Merck, USA) after 6 min. The mixture was thoroughly vortexed and incubated for 10 min. Absorbance was measured at 510 nm using a BioSpectrometer (Eppendorf, Germany). The results were compared to a standard curve prepared with catechin (Sigma-Aldrich, USA), and the total flavonoid content was expressed in milligrams of catechin equivalent per kg of dry weight (mg CE/kg DW) [11-13]

Crystal structure determination by XRD

The sample with the most optimal bioactive components and radical scavenging activity was analyzed using X-ray diffraction (XRD) on its powder form for characterization before extraction. The XRD analysis was conducted with a scan speed of 2°/min, covering a 2θ range of 2.00 - 90.00. The X-ray source was a 2.2 kW Cu anode (40 kV, 40 mA) with a Cu-Kα wavelength of 0.15406 nm.

Morphological analysis using SEM

Morphological analysis was performed on the powder sample with the most optimal radical scavenging activity and bioactive component content using a Scanning Electron Microscope (SEM), model Phenome X5 Pro, operating at an accelerating voltage of 10 kV. The detector used was a SED Full (Secondary Electron Detector) to capture the surface topography of the sample. Prior to observation, the sample was coated with a thin layer of gold (Au) using a Quorum Sputter Coater, with argon as the inert gas.

Functional group analysis using FTIR

FTIR analysis was conducted on the powder and extract samples with the most optimal radical scavenging activity and bioactive components. The infrared spectrum was measured using a Bruker Alpha II FTIR. The spectral resolution used was 4 cm^{-1}, with a spectral range of 600 - 4000 cm^{-1}.

Data analysis

Data were analyzed using Ms. Office and DATAtab software. Antioxidant and bioactive component tests were evaluated with 2-way ANOVA, accounting for blanching treatment and extraction method factors. Bonferroni was applied to identify significant differences at a 95 % confidence level. Additionally, a t-test was used to compare ethanol and distilled water as solvents to assess statistical significance. Pearson's test was performed to analyze correlations between variables.

Results and discussion

The following tables present a summary of the radical scavenging activity (IC_{50}) for DPPH and ABTS, as well as the total phenol, total tannin, and total flavonoid content of *Z. zerumbet* rhizome. **Table 1** summarizes the results using distilled water as a solvent, while **Table 2** summarizes the results using ethanol as a solvent. A 2-way ANOVA was conducted to compare the effects of pretreatment and extraction methods used, as well as their interaction with bioactive components and radical scavenging activity. The results indicate significant differences in the average bioactive component content and antioxidant capacity among different blanching treatments and extraction methods, as denoted by different letters in the mean values. Statistical analysis showed significant interactions ($p < 0.05$) between these 2 factors for radical scavenging activity by the DPPH and ABTS methods, total phenol, total tannin using distilled water and ethanol as solvents, and total flavonoid using ethanol as a solvent, indicating a combined effect of the main factors on the attributes evaluated.

Table 1 Recapitulation of antioxidant capacity (IC_{50}) for DPPH and ABTS, total phenol, total tannin, and total flavonoid content of *Z. zerumbet* rhizome using distilled water as a solvent.

Antioxidant capacity IC_{50} (µg/mL): DPPH					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	1517.13 ± 23.83	748.14 ± 27.27	2477.43 ± 12.79	1242.43 ± 46.27	1496.28 ± 658.44[a]
Blanching-water	1129.17 ± 71.16	453.60 ± 9.81	1184.70 ± 55.54	2196.81 ± 99.88	1241.07 ± 652.56[b]
Blancing-citric acid solution	203.36 ± 11.67	240.03 ± 86.97	421.01 ± 7.00	204.48 ± 15.08	267.22 ± 101.43[c]
Average	949.89 ± 585.79[a]	480.59 ± 225.65[b]	1361.05 ± 900.68[c]	1214.57 ± 864.74[d]	
Antioxidant capacity IC_{50} (µg/mL): ABTS					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	327.97 ± 18.22	247.76 ± 5.14	113.50 ± 1.52	105.51 ± 1.83	198.68 ± 98.12[a]
Blanching-water	299.75 ± 2.60	211.92 ± 1.85	155.57 ± 0.99	147.68 ± 2.67	203.73 ± 63.45[a]
Blancing-citric acid solution	168.01 ± 1.92	187.78 ± 0.47	119.27 ± 1.45	99.27 ± 1.69	143.58 ± 37.34[b]
Average	265.24 ± 74.52[a]	215.82 ± 26.28[b]	129.44 ± 19.78[c]	117.49 ± 22.88[d]	
Total phenol (mg GAE/kg DW)					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	574.09 ± 15.81	109.57 ± 3.54	232.39 ± 12.20	386.24 ± 4.73	326.20 ± 180.91[a]
Blanching-water	910.12 ± 44.66	234.85 ± 19.83	382.53 ± 27.61	421.58 ± 67.53	487.27 ± 267.79[b]
Blancing-citric acid solution	829.79 ± 17.50	179.31 ± 3.77	233.66 ± 9.27	481.80 ± 12.59	431.14 ± 268.47[c]
Average	771.33 ± 154.05[a]	175.41 ± 54.25[b]	282.86 ± 76.41[c]	429.87 ± 54.19[d]	
Total tannin (mg TAE/kg DW)					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	1468.81 ± 168.31	504.65 ± 6.25	798.07 ± 23.92	1102.11 ± 44.83	968.41 ±381.26[a]
Blanching-water	1608.05 ± 56.28	867.95 ± 2.21	1171.41 ± 21.32	1010.74 ± 25.40	1164.54 ± 291.34[b]
Blancing-citric acid solution	1419.45 ± 23.60	460.06 ± 33.85	587.03 ± 15.48	835.92 ± 11.77	825.61 ± 385.42[c]
Average	1498.77 ± 123.24[a]	610.89 ± 194.52[b]	852.17 ± 256.90[c]	982.92 ± 120.08[d]	
Total flavonoid (mg CE/kg DW)					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	1347.14 ± 132.69	310.13 ± 62.84	478.57 ± 76.65	767.16 ± 384.43	725.75 ± 448.78[a]
Blanching-water	1464.69 ± 334.44	422.90 ± 36.04	1366.81 ± 646.83	847.98 ± 97.00	1025.59 ± 538.85[a]
Blancing-citric acid solution	1275.13 ± 758.75	411.98 ± 69.28	447.58 ± 215.80	910.37 ± 232.29	761.26 ± 511.87[a]
Average	1362.32 ± 427.97[a]	381.67 ± 73.58[bc]	764.32 ± 567.51[c]	841.84 ± 238.02[bd]	

Note: Data are presented as mean ± standard deviation (SD). Different letters in each row and each column indicate statistically significant differences between groups. Significance was determined at $p < 0.05$.

Table 2 Recapitulation of antioxidant capacity (IC_{50}) for DPPH and ABTS, total phenol, total tannin, and total flavonoid content of *Z. zerumbet* rhizome using ethanol as a solvent.

Antioxidant capacity IC_{50} (µg/mL): DPPH					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	130.68 ± 4.77	158.40 ± 4.60	97.76 ± 2.21	156.63 ± 1.70	135.87 ± 25.86^{ac}
Blanching-water	206.01 ± 4.24	238.18 ± 6.75	328.83 ± 6.32	105.38 ± 2.19	219.60 ± 83.52^{b}
Blancing-citric acid solution	167.31 ± 9.32	126.86 ± 4.12	109.68 ± 4.30	130.37 ± 4.73	133.56 ± 22.53^{c}
Average	168.00 ± 33.11^{a}	174.48 ± 49.90^{ab}	178.75 ± 112.74^{bc}	130.80 ± 22.36^{d}	
Antioxidant capacity IC_{50} (µg/mL): ABTS					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	69.17 ± 0.79	54.03 ± 0.73	63.62 ± 1.32	63.43 ± 0.22	62.56 ± 5.73^{a}
Blanching-water	93.29 ± 1.02	99.80 ± 0.36	72.36 ± 0.59	97.05 ± 3.44	90.63 ± 11.38^{b}
Blancing-citric acid solution	103.05 ± 2.13	61.05 ± 1.56	71.80 ± 1.15	74.27 ± 0.87	77.54 ± 16.28^{c}
Average	88.50 ± 15.15^{a}	71.62 ± 21.37^{b}	69.26 ± 4.34^{ac}	78.25 ± 14.97^{d}	
Total phenol (mg GAE/kg DW)					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	941.37 ± 15.58	342.52 ± 27.16	114.57 ± 0.58	139.95 ± 13.82	384.60 ± 348.49^{a}
Blanching-water	2107.63 ± 43.93	543.80 ± 74.45	583.48 ± 27.54	2274.69 ± 22.14	1377.40 ± 853.24^{b}
Blancing-citric acid solution	5428.52 ± 269.94	1361.05 ± 161.95	344.96 ± 27.16	1561.07 ± 29.41	2173.90 ± 2025.36^{c}
Average	2825.84 ± 2020.93^{a}	749.12 ± 475.77^{b}	347.67 ± 203.97^{c}	1325.24 ± 941.34^{d}	
Total tannin (mg TAE/kg DW)					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	2749.22 ± 54.68	1156.51 ± 15.09	268.47 ± 9.72	599.03 ± 6.85	1193.31 ± 995.39^{a}
Blanching-water	3142.56 ± 54.80	1729.81 ± 52.09	2328.86 ± 99.51	4047.58 ± 228.19	2812.20 ± 917.35^{b}
Blancing-citric acid solution	7408.52 ± 76.84	3632.39 ± 19.91	2157.53 ± 50.79	2398.96 ± 25.23	3899.35 ± 2195.68^{c}
Average	4433.43 ± 2238.47^{a}	2172.90 ± 1122.78^{b}	1584.95 ± 991.73^{c}	2348.53 ± 1498.16^{d}	
Total flavonoid (mg CE/kg DW)					
	Maseration 24 h	Maseration 48 h	Sonication 15 min	Sonication 30 min	Average
Non blanching	3008.10 ± 21.78	319.94 ± 265.52	104.11 ± 13.88	392.22 ± 33.78	956.09 ± 1247.63^{a}
Blanching-water	4458.85 ± 116.75	1285.39 ± 40.05	1682.00 ± 86.98	4714.04 ± 510.40	3035.07 ± 1645.42^{b}
Blancing-citric acid solution	10590.56 ± 211.66	3657.49 ± 91.45	2474.45 ± 133.68	3652.39 ± 21.25	5093.72 ± 3354.66^{c}
Average	6019.17 ± 3487.73^{a}	1754.27 ± 1494.12^{b}	1420.19 ± 1048.06^{c}	2919.55 ± 1967.17^{d}	

Note: Data are presented as mean ± standard deviation (SD). Different letters in each row and each column indicate statistically significant differences between groups. Significance was determined at $p < 0.05$.

Comparison test between solvent groups

The t-test results reveal significant differences between distilled water and ethanol as solvents for extracting bioactive compounds from *Z. zerumbet* rhizomes. Ethanol consistently outperforms distilled water, as indicated by the high t-values and low *p*-values across all tested parameters. A high absolute t-value reflects a substantial difference between the 2 solvent groups, while a *p*-value lower than 0.001 confirms that these differences are statistically significant. For

antioxidant activity, the IC_{50} values for DPPH and ABTS assays show strong statistical significance (t = 6.6873, $p < 0.001$; t = 8.3065, $p < 0.001$), meaning ethanol extracts exhibit a significantly stronger antioxidant effect.

Tabel 3 Comparison test between solvent groups.

	t	p	Cohen's d
IC_{50} DPPH	6.6873	< 0.001	1.5762
IC_{50}ABTS	8.3065	< 0.001	1.9579
Total Phenol	−3.6562	< 0.001	0.8618
Total Tannin	−5.2867	< 0.001	1.2461
Total Flavonoid	−4.6279	< 0.001	1.0908

Effect size, measured by Cohen's d, quantifies the magnitude of these differences. A Cohen's d greater than 0.8 is considered a large effect, while values above 1.2 indicate a very strong effect. The large effect sizes for IC_{50} values (d = 1.5762 and d = 1.9579) further support ethanol's superior extraction efficiency for antioxidant compounds. Similarly, for total phenol, tannin, and flavonoid content, negative t-values indicate higher concentrations in ethanol extracts (t = −3.6562, t = −5.2867, t = −4.6279; $p < 0.001$). Their corresponding effect sizes (d = 0.8618 to 1.2461) fall within the moderate to large range. These findings highlight the effectiveness of ethanol as a solvent in maximizing the extraction of phenolic and flavonoid compounds, which contribute to the overall antioxidant potential of *Z. zerumbet.*

Radical scavenging activity

To assess the antioxidant capacity of *Z. zerumbet,* 2 methods were utilized: DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid)). Both approaches involve electron transfer and the reduction of a colored oxidizing agent. The DPPH test is based on the reduction of purple DPPH to 1,1-diphenyl-2-picrylhydrazine. Meanwhile, the ABTS test is based in the formation of ABTS blue/green, which can be reduced by antioxidants Borges *et al.* [14] The antioxidant capacity results of *Z. zerumbet* with various pretreatments (blanching, non-blanching, and blanching with 0.05 % citric acid solution) and selected extraction methods (24-h maceration, 48-h maceration, 15-min sonication, and 30-min sonication) using distilled water as a solvent are presented in **Table 1**, while the results using ethanol as a solvent are presented in **Table 2**.

The antioxidant capacity of *Z. zerumbet* rhizome measured by DPPH and ABTS methods with various blanching treatments showed significant differences ($p < 0.05$). *Z. zerumbet* blanching with 0.05 % citric acid exhibited higher antioxidant activity compared to non blanching and blanching without citric acid solution. The highest antioxidant activity for DPPH and ABTS was achieved with citric acid blanching, yielding 267.22 ± 101.43 and 143.58 ± 37.34 µg/mL in distilled water solvent. In ethanol solvent, The DPPH method showed the best antioxidant capacity at 77.54 ± 16.28 µg/mL when citric acid solution was used as a blanching pretreatment. These findings are consistent with studies by Pujimulyani *et al.* [5], Pujimulyani *et al.* [6], which reported increased antioxidant capacity in Curcuma species after citric acid blanching compared to non-acid blanching. Blanching can deactivate polyphenol oxidase, and citric acid can also chelate metals [6]. In the selected extraction method, there were variations in antioxidant activity values measured by DPPH and ABTS. However, it was observed that sonication for 30 min resulted in the highest antioxidant activity compared to 15 min of sonication. The use of ultrasonic waves during sonication facilitates micro cavitation, which breaks down cell walls and accelerates the release of bioactive compounds. As a result, sonication produced extracts with higher antioxidant activity [15].

The difference in antioxidant capacity trends between ethanol and distilled water extracts using maceration and sonication methods occurs due to differences in solvent polarity, which affect the solubility of bioactive compounds. Ethanol is more effective in extracting phenolic and flavonoid compounds, while distilled water tends to extract hydrophilic compounds, leading to variations in antioxidant activity. Additionally, the combination of solvent type and extraction method also contributes to the differences in the obtained results.

The antioxidant capacity of *Z. zerumbet* rhizomes was significantly higher when extracted with ethanol compared to distilled water, as indicated by the lower IC_{50} values from DPPH and ABTS assays ($p < 0.001$), as presented in **Table 3**. This result emphasizes the enhanced antioxidant activity in ethanol extracts. Ethanol's superior ability to extract bioactive compounds, particularly phenolics and flavonoids which play a crucial role in scavenging free radicals and delivering antioxidant effects can be attributed to its capability to dissolve both polar and non-polar compounds effectively. This has been corroborated by several studies. Research consistently shows that ethanol is more effective than distilled water in extracting antioxidants. For example, comparative analyses of different solvent extraction systems have demonstrated that ethanol extracts higher levels of antioxidants from medicinal plants like turmeric, torch ginger, and curry leaves [16]. Additionally, a study on medicinal plants rich in caffeic acid derivatives found that water-ethanol mixtures significantly enhanced antioxidant content compared to water alone [17]. This positive correlation between ethanol-based solvents and higher antioxidant activity, as measured by DPPH and ABTS assays, confirms ethanol's efficacy. This effectiveness can be attributed to ethanol's ability to break down plant cell walls more efficiently, thereby extracting greater quantities of bioactive phenolic acids and flavonoids, which are key contributors to antioxidant properties.

Total phenol

Based on the results of the 2-way ANOVA, the analysis of total phenols in *Z. zerumbet* rhizomes indicated that blanching treatment and extraction method significantly influenced the extraction outcomes, with a notable interaction between the 2 factors. In general, ethanol as a solvent yielded a higher total phenolic content compared to distilled water. According to the t-test results for ethanol and distilled water solvents, a t-value of -3.6562 was obtained for phenol, indicating that ethanol extraction resulted in significantly higher phenol content, with a p-value < 0.001 (**Table 3**). In the distilled water solvent, the average total phenol content ranged from 175.41 ± 54.25 to 771.33 ± 154.05 mg GAE/Kg DW. Meanwhile, in the ethanol solvent, the average total phenol content was higher, ranging from 347.67 ± 203.97 to 2825.84 ± 2020.93 mg GAE/Kg DW. This demonstrates that ethanol is more effective than distilled water in extracting flavonoid compounds from the rhizomes. A similar finding has been widely reported in several studies, including the research by Do *et al*. [18] on *Limnophila aromatica*, which highlighted that the use of ethanol as a solvent was more effective in yielding the highest total phenol content compared to methanol and water Do *et al.* [18] Another study also reported an increase in total phenolic content when ethanol was used as a solvent in root vegetables like white radish, red radish, beet, and carrot [19]. The ability to extract phenolic compounds is strongly influenced by the polarity of the solvent used, the polarity index, and the solubility of phenolic compounds in the solvent. In general, solvents with higher polarity are more effective at extracting larger quantities of polyphenols [20].

The total phenolic content in this study showed the highest average value in the pretreatment with blanching using 0.05 % citric acid as the medium and ethanol as the solvent, with an average of 2173.90 ± 2025.36 mg GAE/kg DW. The increase in total phenolic content is suspected to be due to the degradation of tannins into simpler phenolic compounds [5]. As noted by Kim *et al.* [21] heat treatment of tannic acid can lead to hydrolysis, producing galloyl groups, such as gallotannins, which contribute to the increased phenolic content gallotannins [21]. The selected extraction methods also had a significant effect on the total phenol content based on ANOVA results, with a significance level of $p < 0.05$. The highest mean values were observed in the 24-h maceration and 30-min sonication treatments using ethanol as the solvent, with respective values of 2825.84 ± 2020.93 and 1325.24 ± 941.34 mg GAE/kg DW. Research by Irfan *et al.* [22] demonstrated that both maceration and sonication methods can significantly

enhance total phenolic content in plant extracts, with the efficiency depending on extraction time [22]. Sonication, in particular, is known for improving extraction yields by disrupting plant cell walls through ultrasonic waves, which facilitates the release of phenolic compounds. Previous research on *Cymbopogon citratus* (lemongrass leaves) reported that sonication with ethanol resulted in a higher phenolic content compared to maceration. However, in contrast, this study found that maceration produced a higher total phenolic content than sonication in *Z. zerumbet*. This difference may be influenced by sonication duration, as the study on *C. citratus* applied 60 min of sonication, whereas in this study, only 30 min was used [22]. Sonication time is a crucial factor affecting extraction efficiency, as prolonged sonication can enhance the breakdown of plant cell walls, facilitating the release of phenolic compounds. Therefore, the lower extraction efficiency observed in this study may be attributed to the shorter sonication time, which may have limited the complete release of phenolic compounds from the plant matrix. However, other factors, such as potential differences in the operating parameters of the sonication equipment used, should also be considered as possible contributors to variations in the results.

Total tannin

The total tannin content extracted from *Z. zerumbet* rhizomes using ethanol and distilled water as solvents showed a significant interaction between the blanching treatment and extraction method, based on the variance analysis results. The treatment using ethanol as a solvent yielded the highest average total tannin content compared to distilled water. This was demonstrated by the t-test result, with a t-value of −5.2867, a *p*-value of < 0.001, and a Cohen's d value of 1.2461, indicating a strong effect size. This finding aligns with the study conducted by Hayat *et al.* [23] which investigated *Marrubium vulgare* L. using different solvents [23]. The results showed that total tannin content was higher when extracted using ethanol compared to aqueous. Similar results were also reported by Prasanna and Sridhar in their study on leaf extract of *Hygrophila auriculata* [24]. The total tannin content extracted using aqueous solvent showed lower results compared to ethanol extract, with values of 26.1 and 36.3 mg TAE/g dry material, respectively. Another study comparing the impact of different extraction methods on tannins extracted from *Moroccan Acacia* mollissima bark found that for hydrolysable tannins, ethanol as a solvent under infusion and maceration conditions yielded higher values compared to water as a solvent under the same extraction conditions, with 0.033 ± 0.005 and 0.060 ± 0.001 mg TAE/g bark for water, and 0.070 ± 0.001 and 0.221 ± 0.004 mg TAE/g bark for ethanol. However, in some studies, tannins show optimal extraction results when extracted with water, especially at high temperatures. This may occur because the choice of solvent depends on the extraction method and the type of tannins desired, either condensed or hydrolysable [25].

In this study, it was found that the blanching process could increase the total tannin content extracted from *Z. zerumbet*, using both distilled water and ethanol solvents. This is consistent with the study by Magangana *et al.* [26] which found that blanching reduced enzyme activity and enhanced the extraction of bioactive compounds from pomegranate peel extracts, including tannins [26]. Blanching with citric acid in ethanol showed better results than non-blanching or blanching without agents, with a value of 3899.35 ± 2195.68 mg TAE/kg DW. This aligns with Pujimulyani's research, which demonstrated that condensed tannin content in black and white saffron increased after blanching with 0.05 % citric acid compared to fresh samples [5,6]. It is presumed that condensed tannins are more easily extracted during blanching due to protein denaturation, which releases tannins bound to proteins. Moreover, it is suggested that polyphenol oxidase enzymes in white saffron become inactive after blanching, preventing enzymatic oxidation of tannins [5].

The extraction method has a significant effect on the total tannin content in *Z. zerumbet,* whether using ethanol or water as solvents. Maceration for 24 h yielded the highest results compared to other extraction methods. Maceration for 24 h is considered more optimal than 48 h, likely due to external factors such as prolonged maceration time, which can allow for tannin oxidation or degradation. In the sonication method, sonication for 30 min showed optimal results compared to 15 min of sonication. This is consistent with the study reported by Farahmandfar *et al.* [27] where the results showed that sonication significantly enhanced the

extraction of bioactive compounds of *Arum maculatum* leaves extracts, especially after 40 min of treatment. However, for tannin extraction, 20 min of sonication was found to be optimal [27].

Total flavonoid

Flavonoids are secondary metabolites found in various plant parts, including fruits, herbs, vegetables, and seeds, many of which exhibit therapeutic properties due to their bioactive phytochemical constituents and bioavailability [28]. This study examines the effects of blanching pretreatment, extraction methods, and solvent types. It was found that blanching, whether in ethanol or water, increased total flavonoid content compared to non-blanched extracts. This finding is consistent with Pujimulyani, where a significant increase in flavonoid content after blanching was observed, likely due to the hydrolysis of flavonoid glycosides into aglycones [5]. Blanching also increased flavonoid content threefold in cardoon stalks compared to unblanched samples [29].

The solvent used also affected the amount of total flavonoids extracted. Based on t-test results, ethanol showed the highest average flavonoid content (7408.52 ± 76.84 mg CE/kg DW) with a Cohen's d value of 1.0908. Other studies have reported similar results, such as in *Achillea millefolium* and *Bergenia ciliata*, where ethanol extracts yielded higher flavonoid content compared to aqueous solvents. This is likely because ethanol, due to its intermediate polarity, can dissolve both polar and non-polar compounds, allowing it to extract a broader range of flavonoids, which often contain both hydrophilic and hydrophobic parts. Water, being highly polar, is more limited to extracting hydrophilic compounds, whereas ethanol's ability to interact with various molecular structures makes it more efficient for extracting flavonoids with different solubility properties, leading to higher total flavonoid content [30]. The total flavonoid content is significantly influenced by the extraction method. In this study, 24-h maceration and 30-min sonication yielded the highest flavonoid concentrations, with average values of 1362.32 ± 427.97 and 841.84 ± 238.0 mg CE/kg DW in distilled water, and 6019.17 ± 3487.73 and 2919.55 ± 1967.17 mg CE/kg DW in ethanol Sari *et al.* [31] similarly reported optimal flavonoid extraction from mango leaves using a 1:5 ethanol ratio in maceration. Sonication operates through ultrasonic waves that break cell walls and facilitate mass transfer. Maceration over 24 h is more efficient than 48 h, as extended durations may result in flavonoid degradation or polymerization, thereby reducing extraction efficiency.

Correlation between the test variables of *Z. zerumbet* rhizome

The heatmap illustrates the strength of correlations between bioactive compounds (total phenol, total tannin, and total flavonoid) and antioxidant activity parameters (IC_{50} DPPH and IC_{50} ABTS) in *Z. zerumbet* rhizome extracts. The color gradient ranges from red for strong positive correlations to blue for strong negative correlations.

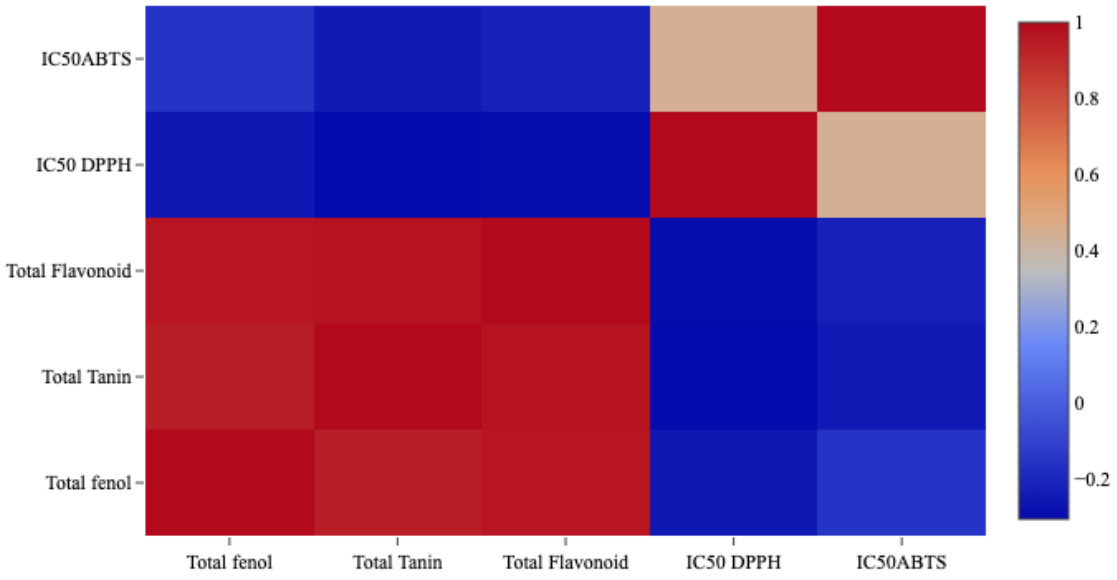


Figure 1 Heatmap showing Pearson correlation analysis between antioxidant capacity, total Phenol, tannin, and total flavoboid.

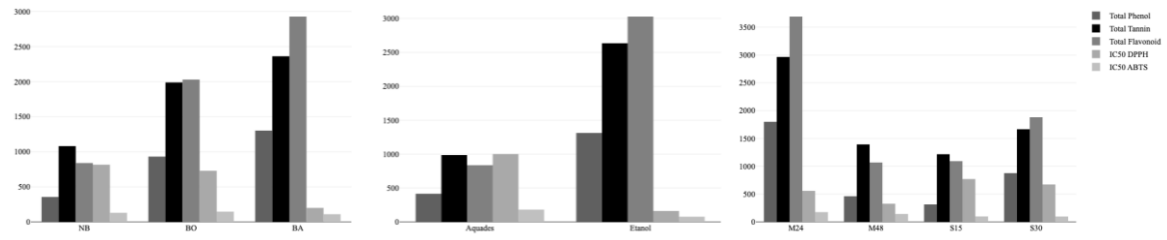
Note: The red blocks represent positive correlations, and the blue blocks represent negative correlations. The colors' shades indicate the strength of the correlations.

Correlation analysis between total phenol, total tannin, total flavonoid, and antioxidant activity measured by IC_{50} DPPH and IC_{50} ABTS showed several important relationships. Total phenol had a strong positive correlation with total tannin ($R = 0.9449$, $p < 0.001$) and total flavonoid ($R = 0.9694$, $p < 0.001$), indicating that increasing phenol content was followed by increasing tannin and flavonoid content. However, total phenol had a weak negative correlation with IC_{50} DPPH ($R = -0.2548$, $p = 0.031$) and a weaker correlation with IC_{50} ABTS ($R = -0.1543$, $p = 0.196$). This negative correlation indicates that the higher the phenol content, the stronger the antioxidant activity (lower IC_{50} value), especially for the DPPH method. Total tannins also showed a strong positive correlation with total flavonoids ($R = 0.9742$, $p < 0.001$) and a negative correlation with IC_{50} DPPH ($R = -0.3035$, $p = 0.01$), which confirmed the role of tannins in enhancing antioxidant activity. The correlation between total tannins and IC_{50} ABTS was weaker ($R = -0.2476$, $p = 0.036$). In addition, total flavonoids also showed a negative correlation with IC_{50} DPPH ($R = -0.2953$, $p = 0.012$) and IC_{50} ABTS ($R = -0.2243$, $p = 0.058$), indicating that flavonoids play an important role in supporting antioxidant activity. A moderate positive correlation between IC_{50} DPPH and IC_{50} ABTS ($R = 0.443$, $p < 0.001$). This suggests that an increase in phenolic content correlates with an increase in tannins and flavonoids, which are known to contribute to the overall antioxidant activity of plant extracts. The negative correlations between these bioactive compounds and IC_{50} DPPH and IC_{50} ABTS demonstrate that as phenol, tannin, and flavonoid concentrations increase, the IC_{50} values decrease, indicating stronger antioxidant activity. Another study also demonstrated a strong correlation between flavonoids and antioxidant activity in chestnuts Martinez *et al.* [32], as well as a positive correlation between phenols and flavonoids. The research further indicated that, in addition to flavonoids, other phenolic compounds also play a role in inhibiting free radicals. Flavonoids are synthesized in plants from aromatic amino acids such as phenylalanine and tyrosine through the shikimate pathway. These compounds play a critical role in antioxidation by donating hydrogen atoms or chelating metals, which contributes to the stabilization of free radicals. The hydrolysis of flavonoid glycosides into their aglycone forms also enhances their antioxidant capacity, as observed in the increased total flavonoid content and antioxidant activity [33]. Additionally, tannins are also

known to contribute to the enhancement of antioxidant activity in *Z. zerumbet.* A similar study was reported by Antasionasti *et al.* [34] which revealed that the extract of nutmeg (*Myristica fragrans*) flesh possesses strong antioxidant activity [34]. The tannin, total flavonoid, and total phenolic contents in nutmeg play a significant role in inhibiting free radicals.

Data visualization for categorizing optimal treatments.

The determination of the best treatment in this study is based on several key factors, including literature review, visualization of the average test results from 5 important variables (total phenol, total flavonoid, total tannin, and antioxidant activity measured using the DPPH and ABTS methods), and correlation analysis between these variables. Based on the correlation analysis, it was found that total tannin, flavonoid, and phenol have a strong positive correlation with each other. Additionally, a significant negative correlation was observed between these 3 bioactive components and antioxidant activity, as measured by the DPPH method using IC_{50} values. This means that the higher the content of bioactive compounds such as phenol, flavonoid, and tannin, the lower the IC_{50} value, indicating stronger antioxidant activity.



(A) (B) (C)

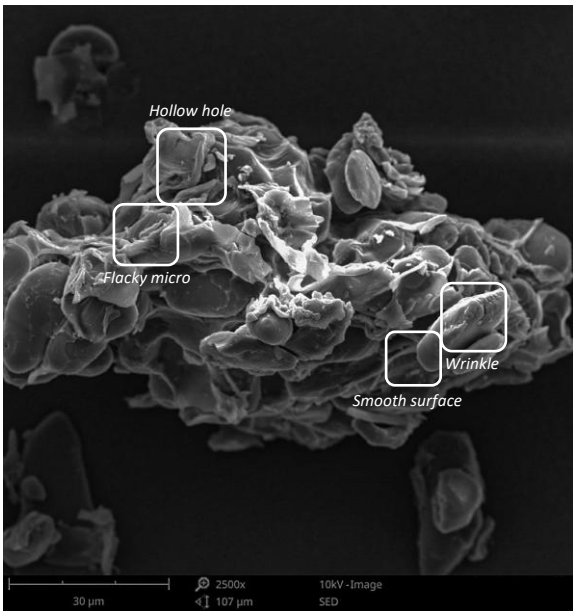
Figure 2 Data visualization based on the average test results.
Note: (A) Pretreatment (NB: Non Blanching, BO: Blanching, BA: Blanching with citric acid 0,05 % solution), (B) Solvent (Ethanol and distilled water), (C) Extraction Method (M24: Maceration 24 h, M48: Maceration 48 h, S15: Sonication 15 min, S30: Sonication 30 min)

The selection of pretreatment using blanching with citric acid solution as a medium is based on a literature review and previous research, including studies by Pujimulyani *et al.* [5], Pujimulyani *et al.* [6], which showed that the use of citric acid can increase the bioactive component content in food materials. Control treatments were used as a comparison to evaluate the effectiveness of citric acid. Based on the average visualization results, blanching with 0.05 % citric acid solution showed the highest average compared to other pretreatment methods, supporting the use of citric acid as the best pretreatment. The selection of solvents was based on the results of the t-test statistical analysis and visualization of the average results comparing the effectiveness of 2 solvents, distilled water and ethanol. The t-test results showed that ethanol was significantly more effective in increasing antioxidant activity and bioactive component content compared to distilled water, which was further supported by the average test result visualization. Ethanol proved to be superior in extracting bioactive components such as phenols, flavonoids, and tannins, as well as producing higher antioxidant activity. The selection of extraction methods was based on the visualization of the average test results of the 5 variables, as well as correlation analysis between the variables. The study results showed that the extraction method using maceration for 24 h and sonication for 30 min provided the most optimal results in increasing bioactive component content compared to other extraction methods. This is in line with extraction theory, where appropriate contact time or sonication energy can maximize the release of bioactive components from plant matrices, thus improving extraction efficiency.

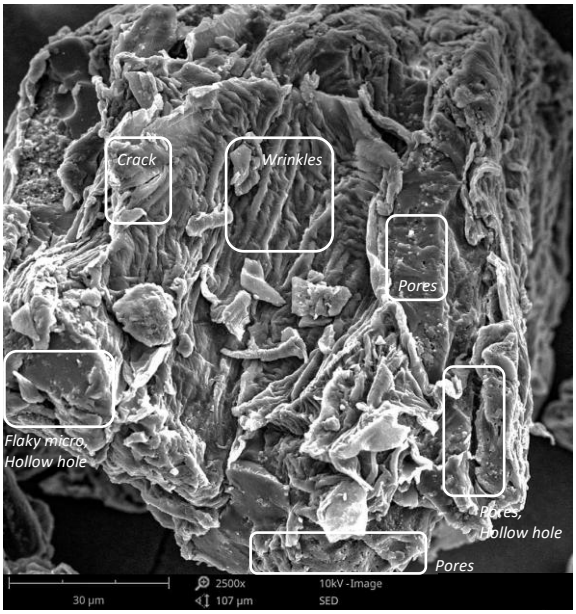
Characteristics of *Z. zerumbet* powder after blanching treatment with 0.05 % citric acid solution.

Scanning electron microscope (SEM)

In the observation using Scanning Electron Microscope (SEM), the morphology of *Z. zerumbet* powder without blanching treatment (**Figure 1(A)**) showed a relatively intact structure with minimal cell wall damage. Flaky micro-particles were identified, which are microscopic fragments formed due to slight cell wall breakage. These particles may have resulted from structural changes during the drying process of the rhizome, leading to cracking or detachment of cell wall components. Additionally, ridges or protrusions on the cell walls were observed, indicating that the structural integrity of the material remained well-preserved. The particle surfaces appeared mostly smooth, suggesting that the powder of the rhizome without blanching pretreatment did not undergo significant deformation, causing bioactive components to remain trapped within the intact cellular structure, thus slowing their release.



(A)



(B)

Figure 3 SEM images of *Z. zerumbet* rhizome powder: (A) non blanching, (B) blanching treatment with 0.05 % citric acid solution.

In contrast, the morphology of *Z. zerumbet* powder that had undergone blanching treatment with 0.05 % citric acid solution (**Figure 1(B)**) showed significant changes, including the formation of numerous pores of varying sizes. These pores, along with hollow holes and spongy formations, indicate that the blanching process caused increased porosity and damage to the cell walls. This facilitated the release of bioactive components, such as antioxidants, by opening the cellular structures and allowing greater access to the internal contents of the particles. The process also showed an increase in the number of flaky micro particles and the presence of ruptures in the cell walls, emphasizing the chemical and physical effects of blanching in increasing the surface area for the extraction of functional components. The significant difference between the morphology of fresh rhizomes and those treated with blanching demonstrates that this treatment not only affects the number of pores but also the texture and overall integrity of the cell walls. The increase in pore number and cavity enlargement in the particles after blanching accelerates the release of bioactive components, such as antioxidants, resulting in higher bioactive activity. Research on green beans with blanching treatment also reported a greater degree of structural damage as blanching temperature increased, along with a reduction in the crystallinity of pectin and cell walls [35].

Crystalline structure

In this X-ray Diffraction (XRD) diffractogram, the diffraction patterns show significant differences between non blanching *Z. zerumbet* powder (A) and powder that has undergone blanching with 0.05 % citric acid solution (B). The non blanching sample exhibits several sharp diffraction peaks, indicating a higher degree of crystallinity. This crystallinity is often associated with a more ordered and tightly packed structure, which can limit the solubility of bioactive compounds.

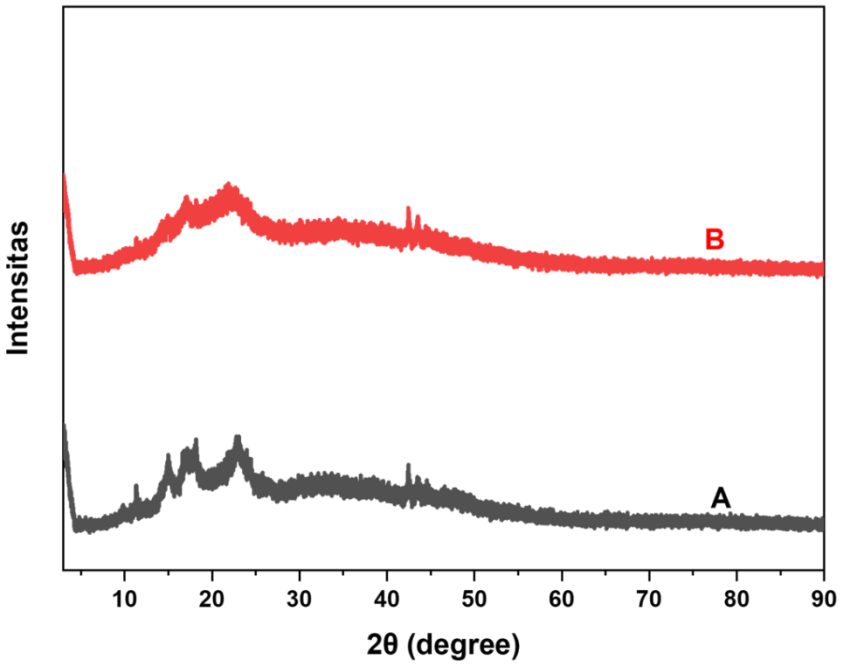


Figure 4 The XRD spectrum of of *Zingiber zerumbet* rhizome powder from: (A) non blanching, (B) blanching treatment with 0.05 % citric acid solution.

In contrast, the XRD pattern for *Z. zerumbet* powder that has undergone blanching (B) shows broader peaks and lower intensity, suggesting a reduction in crystallinity. The blanched sample displays a more amorphous structure, which is typically linked to improved solubility. This increase in amorphous regions can enhance the efficiency of extraction in subsequent processes. With a more open structure after blanching, bioactive compounds such as phenols, flavonoids, and antioxidants are more easily dissolved and released from

the cellular matrix, resulting in higher concentrations of bioactive components in the final extract. For the fresh sample (A), crystalline peaks are observed in the range of 2θ = 10 to 25 °, while in the blanched sample (B), these sharp peaks are replaced with broader, less defined patterns, indicating a transition toward an amorphous structure. This structural transformation is key to improving the extraction yield and bioavailability of active compounds. This study is consistent with research on lyophilized lily flours, where the intensity of the characteristic diffraction peaks decreased, particularly in blanched samples [36]. This indicates that blanching or drying can reduce the relative crystallinity of the samples. This observation is also consistent with the findings of Chen *et al*. [37], who found that the relative crystallinity of blanched samples was lower compared to samples without blanching [37].

FTIR of *Z. zerumbet* powder and extract

In the FTIR spectrum analysis of *Z. zerumbet* samples, functional group changes are clearly observed based on the peak positions and intensity variations following different treatments: Powder of rhizome from non blanching treatment (a), powder of rhizome after blanching with 0.05 % citric acid solution (b), macerated extract of powder of rhizome after blanching with 0.05 % citric acid solution (c), and sonicated extract of powder of rhizome after blanching with 0.05 % citric acid solution (d).

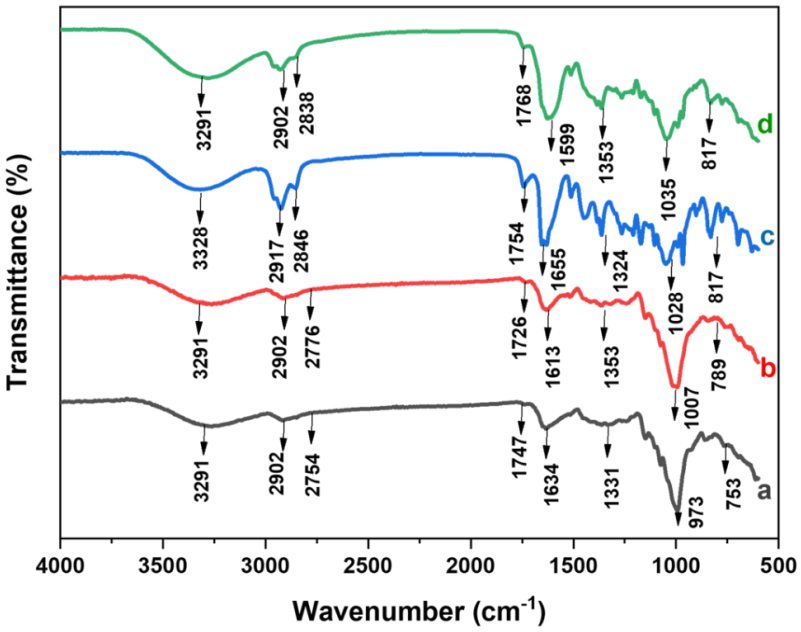


Figure 5 FTIR spectrum of *Z. zerumbet*: Powder of rhizome from non blanching treatment (a), powder of rhizome after blanching with 0.05 % citric acid solution (b), macerated extract of powder of rhizome after blanching with 0.05 % citric acid solution (c), and sonicated extract of powder of rhizome after blanching with 0.05 % citric acid solution (d).

FTIR is an important analytical tool for determining the structural characteristics of products. The FT-IR spectrum is used to identify the functional groups of active components in the extract by analyzing the peak values within the IR radiation range. Based on visual inspection, the FTIR spectra of the powder and extract samples of *Z. zerumbet* showed differences in the number of absorption bands across the evaluated IR range (400 - 4000 cm^{-1}), with the extract sample displaying several new absorption bands. This indicates the presence of other compounds successfully extracted during the treatment process. The infrared spectra of *Z. zerumbet* are shown in **Figure 5**. The spectrum indicates the presence of a broad stretching vibration of the O–H bond in the range of 3200 - 3600 cm^{-1}. Another characteristic band, representing the aromatic skeletal stretching typical of lignans, is observed at 1512 cm^{-1} [38]. All other functional bands have also been documented (**Table 4**).

Table 4 Main functional group bands identified in the FT-IR spectra of sample.

No	Wavenumber (cm^{-1})	Functional group assignment	Comments
1	3200 - 3600	OH stretch	Hydroxyl and phenolic groups in gingerols, shogoals, zingerone, and paradols.
2	2850 - 2970	CH_2 bending/wagging	Backbone of several secondary metabolites like terpenoid and sesquiterpen
3	1640 - 1680	C=C stretching	Terpenes like zingiberene, camphene, β-elemene, and limonene present.
4	1450 -1510	Aromatic skeletal stretch	Aromatic skeletal stretching of lignin.
5	1230 - 1380	C–N stretching	Zingerines.

Conclusions

The research results indicate that blanching *Z. zerumbet* rhizomes with 0.05 % citric acid, combined with ethanol solvent, 24-h maceration, and 30-min sonication, yields optimal outcomes. Blanching treatment enhances both antioxidant activity and the extraction of bioactive compounds, including phenols, flavonoids, and tannins. Ethanol as the extraction solvent significantly improves the yield of these bioactive components, which strongly correlate with antioxidant activity, as confirmed by statistical analysis. The FTIR analysis further supports these findings by confirming the successful extraction of additional functional compounds, indicating structural modifications that enhance bioavailability. Additionally, XRD and SEM analyses reveal structural and morphological changes in the samples, suggesting that blanching and extraction conditions influence the integrity of bioactive compounds. The strong positive correlation between phenols, flavonoids, and tannins suggests that these compounds collectively contribute to the enhanced antioxidant properties observed. This study underscores the importance of extraction methods and pretreatment in maximizing the therapeutic potential of Z. zerumbet as a natural antioxidant source and demonstrates the effectiveness of citric acid blanching combined with ethanol extraction in enhancing bioactive yields.

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