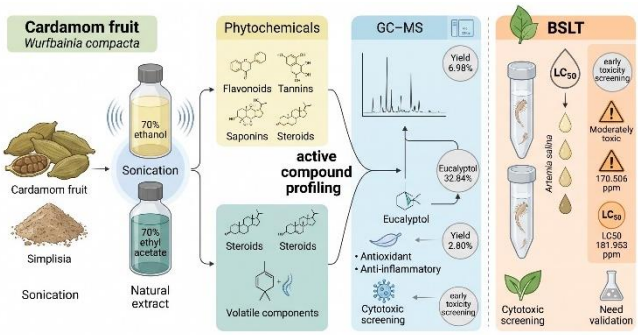
***JKPK (JURNAL KIMIA DAN PENDIDIKAN KIMIA)**, Vol. 11, No. 2, 2026* *pp. 304-319*
Chemistry Education Study Program, Universitas Sebelas Maret *ISSN 2503-4146*
https://jurnal.uns.ac.id/jkpk *ISSN 2503-4154 (online)*

Toxicity and Active Compound Content of Cardamom Fruit Extract (*Wurfbainia compacta Sol. ex Maton*)

Nur Nunu Prihantini[1]*, Fransiska Sitompul[2], Erica Gilda Misnawati Simanjuntak[3], Berlian Feodora Pangaribuan[4]

[1]Department of Medical Biochemistry, Faculty of Medicine, Universitas Kristen Indonesia, Jakarta, Indonesia
[2]Department of Pharmacology & Therapy, Faculty of Medicine, Universitas Kristen Indonesia, Jakarta, Indonesia
[3]Department of Anesthesiology, Faculty of Medicine, Universitas Kristen Indonesia, Jakarta, Indonesia
[4]Clinical Student, Faculty of Medicine, Universitas Kristen Indonesia, Jakarta, Indonesia

ABSTRACT



Cardamom (*Wurfbainia compacta* Sol. ex Maton), a member of the Zingiberaceae family, contains various secondary metabolites with potential biological activities. This study aimed to determine the active compound profile and toxicity of cardamom fruit extracts prepared using 70% ethanol and 70% ethyl acetate. Extraction was performed by sonication, followed by phytochemical screening using the Harborne method and compound identification by gas chromatography–mass spectrometry (GC–MS). Toxicity was evaluated using the Brine Shrimp Lethality Test (BSLT) to determine the LC_{50} values. The extraction yields were 6.98% for the 70% ethanol extract and 2.80% for the 70% ethyl acetate extract. Phytochemical screening showed that the 70% ethanol extract contained flavonoids, tannins, saponins, and steroids, whereas the 70% ethyl acetate extract contained steroids. GC–MS analysis identified several volatile and semi-volatile compounds in both extracts. The BSLT results showed LC_{50} values of 170.506 ppm for the 70% ethanol extract and 181.953 ppm for the 70% ethyl acetate extract, placing both extracts in the moderately toxic category. The 70% ethanol extract showed a broader phytochemical profile and slightly greater toxicity than the 70% ethyl acetate extract. These findings support further investigation of cardamom fruit extracts for their biological activity and safety.

Keywords: *brine shrimp lethality test; cardamom fruit; phytochemical screening; toxicity.*

**Corresponding Author:* *nunu.prihantini@uki.ac.id*

How to cite: N. N. Prihantini, F. Sitompul, E. G. M. Simanjuntak, and B. F. Pangaribuan, "Toxicity and Active Compound Content of Cardamom Fruit Extract (*Wurfbainia compacta* Sol. ex Maton)," *Jurnal Kimia dan Pendidikan Kimia (JKPK)*, vol. 11, no. 2, pp. 304–319, 2026. [Online]. Available: https://doi.org/10.20961/jkpk.v11i2.116919
Received: 2026-03-29 *Accepted: 2026-08-02* *Published: 2026-08-31*

INTRODUCTION

Indonesia is recognized as one of the world's megadiverse countries and possesses abundant plant resources that have long been used in traditional medicine. Cardamom (Wurfbainia compacta Sol. ex Maton), a member of the Zingiberaceae family, is widely used both as a spice and as an ingredient in traditional medicinal preparations. Traditional uses of cardamom include the management of digestive disorders, inflammation, and infectious

diseases, reflecting its broad pharmacological potential [1].

Cardamom's pharmacological activity is closely associated with its secondary metabolite composition. Previous studies have reported the presence of bioactive compounds such as flavonoids, polyphenols, saponins, alkaloids, steroids, and terpenoids in cardamom fruit [2]. Gas chromatography–mass spectrometry (GC–MS) analysis has also identified major volatile constituents, including 1,8-cineole and α-terpineol [1]. These compounds have been associated with several biological activities, including anti-inflammatory, antioxidant, antimicrobial, and potential cytotoxic effects.

Previous investigations have also identified other bioactive constituents in cardamom, including borneol, caryophyllene, camphor, and humulene. Several of these compounds have been reported to exhibit anti-inflammatory potential through modulation of inflammatory mediators such as IL-6 and TNF-α [3]. Flavonoids and vitamin C present in cardamom may also contribute to antioxidant activity by scavenging free radicals and reducing oxidative cellular damage [4]. These findings support the potential of cardamom as a natural source of bioactive compounds for the development of herbal preparations.

Safety evaluation is an essential component in the development of medicinal plant extracts because pharmacological potential must be accompanied by evidence regarding toxicity and safe levels of use. Toxicity testing provides preliminary information on the biological effects of an extract, particularly when it is intended for further development as a pharmaceutical product or health supplement. Previous toxicity assessment using the Brine Shrimp Lethality Test (BSLT) reported an LC_{50} value of 26.60 ppm for cardamom fruit extract, indicating considerable toxicity toward Artemia salina and suggesting potential cytotoxic activity that warrants further investigation [5].

Evidence from acute toxicity studies also indicates that cardamom extract may affect physiological functions at certain doses, including liver-related parameters associated with the metabolism of xenobiotic substances [6]. These findings emphasize the importance of evaluating the toxicity profile of cardamom extract alongside its chemical composition. Existing studies have largely focused on either phytochemical constituents, biological activities, or toxicity independently, whereas information integrating the active compound profile with toxicity characteristics remains limited. Such integrated information is important for assessing the potential of cardamom as a safe and effective candidate for phytopharmaceutical development.

The present study aimed to evaluate the toxicity and active compound profile of cardamom fruit extract (Wurfbainia compacta Sol. ex Maton). The study specifically examined phytochemical constituents and GC–MS-detected compounds in 70% ethanol and 70% ethyl acetate extracts and assessed their toxicity using the Brine Shrimp Lethality Test. The findings are expected to provide scientific information that may support the further development and safety evaluation of cardamom-derived herbal preparations.

METHODS

This study employed an in vitro experimental laboratory design. All phytochemical screening and GC–MS analyses were conducted at the Biopharmaceutical Laboratory, IPB University, while extraction procedures were performed at the Biochemistry Laboratory and Biopharmaceutical Laboratory of IPB University. The study was conducted from September to December 2025.

1. Materials and Reagents

The plant material used in this study was cardamom fruit (*Wurfbainia compacta* Sol. Ex Maton) obtained from traditional markets in Jakarta, Indonesia. Botanical identification and authentication of the plant specimen were carried out at the Plant Taxonomy and Characterization Laboratory, National Research and Innovation Agency.

The chemicals and reagents used included 70% ethanol, 70% ethyl acetate, ammonia solution (NH_3), chloroform ($CHCl_3$), sulfuric acid (H_2SO_4 2 M), Dragendorff reagent, Mayer reagent, Wagner reagent, distilled water, magnesium powder, hydrochloric acid–ethanol solution (HCl:EtOH, 1:1), amyl alcohol, ferric chloride ($FeCl_3$ 10%), hot ethanol, diethyl ether, concentrated sulfuric acid, acetic anhydride ($(CH_3CO)_2O$), methanol (MeOH), sodium hydroxide (NaOH 10%), potassium dichromate ($K_2Cr_2O_7$), sodium chloride (NaCl), dimethyl sulfoxide (DMSO 2%), and *Artemia salina* Leach larvae.

2. Instruments

The instruments used in this study included analytical balances, pipettes, volumetric pipettes, Erlenmeyer flasks, magnetic stirrers, water baths, ovens, blenders, filter paper, micropipettes, test tubes, test tube racks, aerators, thermometers, microplates, and hatching chambers. GC–MS analysis was performed using an Agilent Technologies 7890B Gas Chromatography system coupled with a 5977A Mass Selective Detector (MSD) equipped with an autosampler.

3. Preparation of Simplicia and Extraction

Fresh cardamom fruits were washed with running water to remove adhering impurities and then air-dried at room temperature for 24 h. The samples were subsequently dried in a hot-air oven at 40–45°C until a constant weight was achieved. The dried samples were ground into powder using a laboratory blender and sieved to obtain uniform particle size simplicia.

A total of 400 g of powdered cardamom simplicia was divided into two 200-g portions. Each portion was extracted separately using 70% ethanol or 70% ethyl acetate. Extraction was performed using the sonication-assisted extraction method with a solvent-to-sample ratio of 10:1 (v/w). The extraction mixture was sonicated in an ultrasonic bath operating at 40 kHz and 150 W for 30 min at room temperature (28 ± 2°C). The extraction process was repeated three times to maximize compound recovery. After each extraction cycle, the mixture was filtered using Whatman No. 1 filter paper, and the filtrates were combined.

The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C until a viscous

extract was obtained. The concentrated extract was further dried in a water bath at 40°C to remove residual solvent. The extraction yield was calculated based on the dry weight of the extract relative to the initial simplicia weight. All extraction procedures were performed in triplicate.

4. Phytochemical Screening

Preliminary phytochemical screening was conducted using the Harborne method to qualitatively identify the presence of alkaloids, flavonoids, tannins, saponins, triterpenoids/steroids, and hydroquinones in the extracts.

Qualitative phytochemical screening was conducted using specific color and precipitation reactions for alkaloids, flavonoids, tannins, saponins, triterpenoids/steroids, and hydroquinones [7]. Flavonoids were identified using the Shinoda test with magnesium powder and HCl, indicated by red or orange coloration. Tannins were detected using $FeCl_3$ solution, producing dark blue or greenish-black coloration. Saponins were identified by the formation of stable foam after vigorous shaking. Triterpenoids and steroids were detected using the Liebermann–Burchard reaction, characterized by reddish-brown or green-blue coloration. Hydroquinones were identified using NaOH solution, indicated by the formation of yellow to red coloration. All phytochemical tests were performed in triplicate.

5. GC–MS Analysis

Chemical constituents of the cardamom fruit extracts were analyzed using GC–MS. Separation was performed using an HP-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector temperature was maintained at 250°C with a split ratio of 1:10.

The oven temperature program was set as follows: initial temperature 50°C held for 2 min, increased to 150°C at 10°C/min, followed by an increase to 280°C at 5°C/min and held for 10 min. The total run time was approximately 45 min. The MS detector operated under electron ionization mode at 70 eV with a scan range of m/z 40–550. The ion source temperature was maintained at 230°C.

Identification of chemical compounds was performed by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) library database. Compounds with similarity index values ≥90% were considered positively identified. Relative compound abundance was determined based on peak area normalization. GC–MS analysis was performed in triplicate.

6. Brine Shrimp Lethality Test (BSLT)

Toxicity testing was carried out using the Brine Shrimp Lethality Test (BSLT) method with *Artemia salina* Leach larvae. *Artemia salina* eggs were hatched in artificial seawater prepared from NaCl solution under continuous aeration and illumination for 48 h at room temperature. Active nauplii were collected and used for testing.

Extract samples were dissolved in 2% DMSO and diluted to obtain concentrations of 62.5, 125, 225, 500, 1000 ppm. For each

concentration, 10 larvae were transferred into test vials containing 5 mL of sample solution. Negative controls contained seawater and 2% DMSO without extract. Each treatment and control was conducted in triplicate. After 24 h of exposure, the number of dead larvae was counted, and mortality percentage was calculated. The LC50 value was determined using probit analysis to evaluate the toxicity level of the extracts.

RESULTS AND DISCUSSION

1. Taxonomic Identification and Yield of 70% Ethanol and 70% Ethyl Acetate Extracts of Cardamom Fruits

Taxonomic identification conducted by the National Research and Innovation Agency (BRIN) confirmed the plant material as *Wurfbainia compacta* Sol. ex Maton. The morphological appearance of the identified cardamom plant and fruit is presented in Figure 1.

(a) (b)

Figure 1. Cardamom Tree (a) and Fruit (b)
Source: Author's Documentation

The yield produced from 70% ethanol and 70% ethyl acetate extracts of cardamom fruit can be seen in Table 1. The yield value of making 70% ethanol extract of cardamom fruit was 6.98% while the 70% ethyl acetate extract of cardamom fruit was 2.80%. Yield is the ratio of the dry weight of the extraction product to the weight of the raw material used. A high yield indicates a greater amount of bioactive components in the material. The higher the yield, the more compounds were successfully extracted from the extracted natural material [8].

Table 1. Percentage yield of 70% ethanol extract and 70% ethyl acetate extract of cardamom fruit

No	Test Material	Weight of Simplicia (g)	Weight of Extract (g)	Yield (%)
1.	70% ethanol extract	200	13.95	6.98%
2.	70% ethyl acetate extract	200	5.60	2.80%

Extraction of cardamom fruit with 70% ethanol produced a yield of 6.98%. According to the Indonesian Herbal Pharmacopoeia (FHI), ethanol is a common solvent in the extraction process of herbal medicines because it is relatively safe, evaporates easily, and has good ability to dissolve various classes of secondary metabolites. The water content in 70% ethanol plays an important role in the development of plant cell walls (swelling effect), thus facilitating the solvent's penetration into the herbal medicine tissue and optimally dissolving the active compounds [8]

Extraction of cardamom fruit with 70% ethyl acetate produced a yield of 2.80%. This yield is within the typical range for semi-polar cardamom fractions or extracts, which is around 1-4%. Although FHI does not set specific standards for the yield of cardamom extract with ethyl acetate solvent (2.80%), it shows that the extraction process is able to

extract semi-polar active compounds from raw materials well and according to the extraction characteristics of ethyl acetate solvent [9]

2. Phytochemical Testing of Cardamom Fruit Extract

The results of the phytochemical test of cardamom fruit extract are shown in Table 2. The 70% ethanol extract of cardamom fruit showed positive results for flavonoids, steroids, saponins, and tannins. The 70% ethyl acetate extract of cardamom fruit showed positive results for steroids.

Table 2. Phytochemical test results of cardamom fruit extract

No.	Compound Groups	Observed	
		Ethanol 70%	Ethyl Acetate 70%
1.	Alkaloids		
	a. Dragendorff	–	–
	b. Mayer	–	–
	c. Wagner	–	–
2.	Flavonoids	+	–
3.	Triterpenoids	–	–
4.	Steroids	+	+
5.	Saponins	+	–
6.	Tannin	+	–
7.	Hydroquinone	–	–

Description:
(–) : Not detected
(+) : Detected

The difference in phytochemical content between the two extracts is influenced by the type and polarity of the solvent used. 70% ethanol is a semi-polar solvent with a relatively high water content, making it capable of dissolving polar to semi-polar compounds such as flavonoids, tannins, and saponins [10]. 70% ethyl acetate is semi-polar with a tendency to be more non-polar than ethanol, making it more selective in extracting compounds with medium polarity, such as steroids. This explains why steroids were detected in the 70% ethyl acetate extract [11], [12].

The broader range of phytochemical classes detected in the 70% ethanol extract was consistent with previous reports showing the presence of flavonoids, tannins, saponins, and steroids in cardamom extracts [13]. Differences in phytochemical composition between extracts may arise from differences in solvent polarity and the affinity of individual secondary metabolites for the extraction medium. The present findings therefore indicated that 70% ethanol extracted a wider variety of detectable phytochemical classes from cardamom fruit than 70% ethyl acetate under the conditions used in this study [14].

3. Identification of Cardamom Fruit Extract with GCMS

The GC-MS chromatogram of 70% ethanol extract of cardamom fruit yielded five peaks. Of these, two peaks were identified with the highest similarity values, as shown in Figure 2.

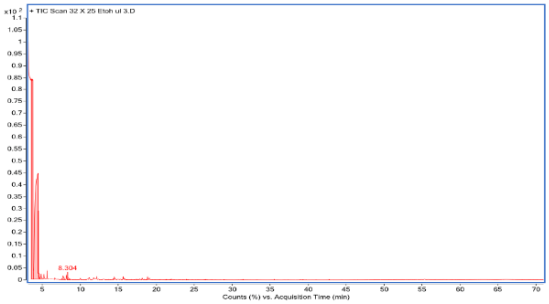


Figure 2. GC-MS chromatogram of 70% ethanol extract of cardamom fruit

Table 3. GC-MS analysis of 70% ethanol extract of cardamom fruit

Retention Time (min)	Area (%)	Similarity	Compounds Name
7.714	0.78	95.24	p-Xylene
4.83	0.78	90.56	1,3,5-Cyclohepatriene

The results of the identification of compounds detected in the 70% ethanol extract of cardamom fruit are presented in Table 3. Based on the results of the GC-MS analysis of the 70% ethanol extract of cardamom fruit, two main compounds were identified based on the percentage (%) area as a parameter of relative abundance. The p-xylene compound was detected at a retention time of 7.714 minutes with an area percentage of 0.78% and a similarity level of 95.24%. The 1,3,5-cycloheptatriene compound was detected at a retention time of 4.83 minutes with an area percentage of 0.78% and a similarity level of 90.56%.

The results of the GC-MS chromatogram of 70% ethyl acetate extract of cardamom fruit obtained 29 peaks, of which there were 12 highest peaks based on the similarity value that were successfully identified and can be seen in Figure 3 and Table 4.

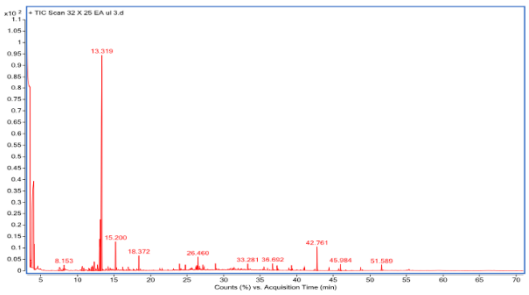


Figure 3. GC-MS chromatogram of 70% ethyl acetate extract of cardamom fruit

Table 4. GC-MS analysis of 70% ethyl acetate extract of cardamom fruit

No	Retention Time (min)	Area (%)	Similarity	Name of Compound
1	13.319	32.84	98.76	Eucalyptol
2	13.031	4.23	95.64	Benzene, 1-methyl-3-(1-methylethyl)-
3	4.078	0.58	95.26	3-Penten-2-one, (E)
4	7.539	1.33	94.32	o-Xylene
5	26.46	0.95	93.58	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha., 7.alpha., 8a.beta.)]
6	10.623	0.83	93.49	Cyclohexene, 4-ethenyl-1,4-dimethyl
7	42.761	1.62	92.89	Eicosane
8	36.692	0.66	92.75	n-Hexadecanoic acid
9	45.984	0.51	91.50	Heneicosane
10	12.73	0.76	91.32	Bicyclo[3.1.1]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha., 2.alpha.,5.alpha) alpha-Terpineol
11	18.372	1	91.04	4-Cyclopentene-1,3-dione
12	8.153	0.47	91.00	

The GC–MS analysis of the 70% ethyl acetate extract of cardamom fruit showed that the eucalyptol compound was detected at a retention time of 13.319 minutes with the highest area percentage of 32.84%, making it a major component in the extract. The benzene compound, 1-methyl-3-(1-methylethyl)- was detected at a retention time of 13.031 minutes with an area percentage of 4.23%. The eicosane compound appeared at

a retention time of 42.761 minutes with an area percentage of 1.62%, followed by o-xylene at a retention time of 7.539 minutes with an area percentage of 1.33%. The alpha-terpineol compound was detected at a retention time of 18.372 minutes with an area percentage of 1.00%, while the naphthalene derivative compound was detected at a retention time of 26.460 minutes with an area percentage of 0.95%. Other compounds identified with relatively low area percentages include cyclohexene derivatives at a retention time of 10.623 minutes at 0.83%, bicyclo[3.1.1]hexan-2-ol derivatives at a retention time of 12.730 minutes at 0.76%, n-hexadecanoic acid at a retention time of 36.692 minutes at 0.66%, 3-penten-2-one (E-) at a retention time of 4.078 minutes at 0.58%, heneicosane at a retention time of 45.984 minutes at 0.51%, and 4-cyclopentene-1,3-dione at a retention time of 8.153 minutes at 0.47%, which indicates that these compounds are minor components in the extract.

Results of GC–MS analysis of 70% ethanol extract of cardamom fruit revealed several volatile compounds with varying retention times. The detected compounds included 1,3,5-Cycloheptatriene, p-Xylene, and 4-Cyclopentene-1,3-dione, with similarity levels to the 2017 NIST database of 90.56%, 95.24%, and 78.11%, respectively. These similarity values indicate good compound identification, effectively representing the volatile chemical components contained in the 70% ethanol extract of cardamom fruit [1]. 1,3,5-Cycloheptatriene and p-Xylene belong to the relatively volatile aromatic hydrocarbon group, enabling optimal detection using the GC–MS method. The presence of these aromatic compounds aligns with the solvent characteristics of ethanol, which is capable of dissolving semi-polar to non-polar compounds. The identified compound, 4-Cyclopentene-1,3-dione, belongs to the carbonyl compound group, known to act as an intermediate in the biosynthesis pathway of secondary metabolites in plants [1], [15] The correlation between GC–MS analysis results and phytochemical testing of 70% ethanol extract of cardamom fruit indicates the presence of flavonoids, steroids, saponins, and tannins. The differences in results obtained from the two analytical methods, GC–MS and phytochemistry, are related to differences in working principles and the range of compounds identified.

The GC–MS method is more effective in detecting volatile and semi-volatile compounds with relatively low molecular weights and heat stability. Secondary metabolites such as flavonoids, tannins, saponins, and steroids are generally non-volatile, have large molecular weights, and are thermolabile, so they cannot be optimally detected using GC–MS without prior derivatization. This condition results in compounds detected in phytochemical tests not appearing directly in the GC–MS analysis results. The presence of volatile compounds identified by GC–MS reflects the complexity of the chemical composition of the 70% ethanol extract of cardamom fruit. The detected aromatic and carbonyl compounds have the potential to contribute to the biological activity of the extract, either directly or through synergistic interactions with non-

volatile secondary metabolites confirmed in phytochemical tests [16].

The relationship between GC–MS results and phytochemical tests from this study also aligns with the generalization in the literature that volatile/semi-volatile compounds detected by GC–MS differ from non-volatile secondary metabolites, such as flavonoids or saponins, found in phytochemical tests but not directly detected by GC–MS without derivatization. These results have been reported in other studies using GC–MS for phytochemical characterization of plant extracts, where volatile component profiles vary depending on tissue type, extraction technique, and analytical method used [17].

GC–MS analysis of 70% ethyl acetate extract of cardamom fruit identified 12 compounds with varying retention times, area percentages, and similarity levels. The compound with the highest area percentage was eucalyptol at 32.84% with a similarity value of 98.76%, indicating that it is the dominant component in the 70% ethyl acetate extract. Other identified compounds included benzene, 1-methyl-3-(1-methylethyl)-, o-xylene, α-terpineol, and several hydrocarbon compounds and terpene derivatives, which are generally volatile to semi-volatile [1].

The dominance of eucalyptol, a member of the oxygenated monoterpene group, reflects the ability of 70% ethyl acetate to dissolve semi-polar compounds, particularly essential oil components. These characteristics align with ethyl acetate's properties as a semi-polar solvent, effectively extracting terpenoids and their derivatives. The presence of α-terpineol further strengthens the indication of terpene components in the extract, although qualitatively, triterpenoids were not detected in phytochemical tests [18].

Phytochemical test results on 70% ethyl acetate extract of cardamom fruit showed positive detection of steroid compounds. The difference in findings between phytochemical tests and GC–MS analysis is related to the different working principles of each method. Phytochemical tests are qualitative and identify compound groups based on specific reactions, while GC–MS focuses on identifying individual compounds that are volatile and heat-stable [19].

Steroid compounds detected in phytochemical tests are generally non-volatile and have relatively large molecular weights, making them undetectable directly through GC–MS analysis without derivatization. These characteristics prevent steroid compounds from appearing in GC–MS chromatograms, even though they have been qualitatively confirmed through phytochemical tests. The compound profile identified by GC–MS in the 70% ethyl acetate extract of cardamom fruit is dominated by volatile and semi-volatile compounds, such as monoterpenes and aromatic hydrocarbons. The results of GC–MS analysis and phytochemical tests on the 70% ethyl acetate extract of cardamom fruit provide a complementary picture of the extract's chemical composition. GC–MS analysis plays a role in identifying volatile and semi-volatile compounds that potentially contribute to the extract's biological activity,

while phytochemical tests confirm the presence of non-volatile secondary metabolites, namely steroids, which also have potential pharmacological activity. The combination of these two analytical methods strengthens our understanding of the complexity of the chemical composition of the 70% ethyl acetate extract of cardamom fruit [20].

The results of the GC–MS analysis of 70% ethyl acetate extract of cardamom fruit in this study indicate agreement with several previous studies that reported that ethyl acetate solvent tends to extract volatile to semi-volatile compounds, especially terpenes and their derivatives. Previous studies on the GC–MS analysis of ethyl acetate extracts of aromatic plants and spices reported the dominance of monoterpene compounds such as eucalyptol, α-terpineol, and aromatic hydrocarbon compounds, which act as the main components of essential oils and volatile fractions of plants. These findings are in line with the results of the study that showed eucalyptol as the compound with the highest area percentage, thus indicating that 70% ethyl acetate is effective in extracting volatile semi-polar components. Differences in the composition and proportion of detected compounds compared to previous studies may be influenced by variations in plant species, plant parts used, geographical conditions, extraction methods, and GC–MS analysis parameters. The similarity of the detected volatile compound profiles strengthens that the results of this study are consistent with the characteristics of ethyl acetate extracts as reported in previous literature [21].

4. Cardamom Fruit Extract Toxicity Test

The results obtained from the mortality data for Artemia salina L. larvae are shown in Table 5, and the linear regression equation for the relationship between extract concentration and the probit value of shrimp larvae is shown in Figure 4. The BSLT test results showed that 17 larvae died at a concentration of 225 ppm, resulting in the LC50 value of the 70% ethanol extract being 170.506 ppm

Table 5. Observation results of the death of Artemia salina L. larvae in 70% ethanol extract of cardamom fruit

Concentration (ppm)	Number of Larvae	Number of Dead Larvae			Number of Deaths in All Replications	% of Deaths in All Replications
		1	2	3		
Control (0)	10	0	0	0	0	0
62.5	10	2	2	1	5	16.6
125	10	4	3	4	11	36.6
225	10	6	5	6	17	56.6
500	10	9	8	8	25	83.3
1000	10	10	10	10	30	100

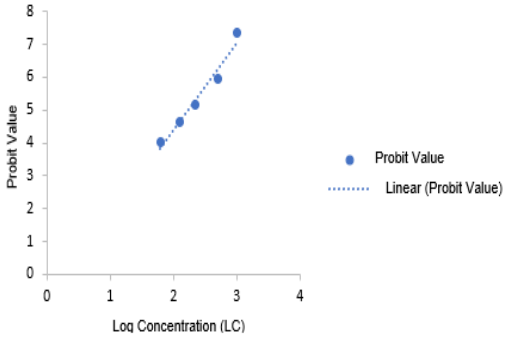


Figure 4. Graph of toxicity test results of 70% ethanol extract of cardamom fruit

Based on the research results, data on the mortality of *Artemia salina* L. larvae were obtained as in Table 6 and the linear regression equation for the relationship between extract concentration and the probit value of shrimp larvae was in Figure 5. The results of the BSLT test showed that the mortality of larvae at a concentration of 225 ppm was 20 larvae, so the LC50 value of 70% ethyl acetate extract was 181.953 ppm.

Table 6. Observation results of the death of Artemia salina L. larvae in 70% ethyl acetate extract of cardamom fruit

Concentration (ppm)	Number of Larvae	Number of Dead Larvae			Number of Deaths in All Replications	% of Deaths in All Replications
		1	2	3		
Control (0)	10	0	0	0	0	0
62.5	10	1	0	0	1	3.33
125	10	4	4	3	11	36.67
225	10	8	6	6	20	66.67
500	10	10	9	10	29	96.67
1000	10	10	10	10	30	100

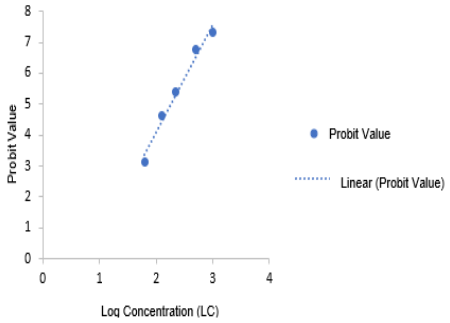


Figure 5. Graph of toxicity test results of 70% ethyl acetate extract of cardamom fruit

Toxicity testing uses the Brine Shrimp Lethality Test (BSLT) as an initial approach to assess the cytotoxic potential of an extract. High toxicity to Artemia salina larvae is often associated with the compound's ability to inhibit growth or cause cell death, including cancer cells. Several studies have shown a correlation between BSLT toxicity test results and cytotoxic activity in cancer cell cultures, making this method relevant as an initial step in screening candidate anticancer compounds [22]. Toxicity testing plays a role not only in assessing safety aspects but also plays a crucial role in identifying the anticancer potential of plant extracts. The results of this study provide a scientific basis for further research using in vitro and in vivo cytotoxic tests on cancer cells, as well as for more in-depth studies of the biological mechanisms of action [23]. Toxicity testing on a 70% ethanol extract of cardamom fruit yielded an LC50 value of 170.506 ppm. According to the classification of Mayer et al. The LC50 value of 70% ethanol extract of cardamom fruit is categorized as moderate

toxic (LC50 between 100-500 ppm). The LC50 value indicates that 70% ethanol extract of cardamom fruit has a fairly strong level of toxicity, thus indicating the presence of secondary metabolites that can provide cytotoxic effects, which is an important characteristic in the development of anticancer drug [5], [24]

The probit analysis produced a linear regression equation of y = 2.6495x – 0.913 with a coefficient of determination (R^2 = 0.9658). The R^2 value, which is close to 1, indicates a very strong relationship between the log concentration and the probit value of larval mortality, making the data reliable for determining the LC50 value. Based on the regression equation, the LC50 value was 170.506 ppm, categorizing the 70% ethanol extract of cardamom fruit as toxic according to Meyer et al.'s criteria, which state that an extract is toxic if its LC50 is ≤ 1000 ppm. This toxic nature indicates the presence of bioactive compounds in the 70% ethanol extract of cardamom fruit, which can cause physiological disturbances in Artemia salina larvae, such as cell membrane damage, nervous system disorders, and metabolic inhibition [24].

The toxic properties of the 70% ethanol extract of cardamom fruit are thought to be closely related to the detected phytochemical compounds. Flavonoids and tannins are known to disrupt cell membrane permeability and inhibit enzyme activity, thus causing metabolic disorders and the death of Artemia salina larvae. Saponins act as surfactants that can damage cell membrane structures, while steroids have the potential to affect membrane stability and physiological cell function. The combination of these compounds is suspected to have a synergistic toxic effect on test organisms [25].

Aromatic compounds such as p-xylene and cycloheptatriene are known to have biological activity that can disrupt the nervous system and cellular respiration of small organisms. Furthermore, 4-cyclopentene-1,3-dione has a reactive carbonyl group that has the potential to interact with cellular proteins and enzymes. The presence of these compounds supports the results of toxicity tests, where a 70% ethanol extract of cardamom fruit demonstrated toxic activity against Artemia salina larvae [26].

The toxicity results of the 70% ethanol extract in this study align with several previous studies that reported that ethanol extract of cardamom fruit has strong biological activity against test organisms. Previous research indicated that ethanol extract of cardamom can cause moderate to strong toxic effects in the BSLT test, which is associated with the content of flavonoids, phenolics, and volatile aromatic compounds. The differences in reported toxicity levels between studies are likely influenced by differences in extraction methods, the concentration of ethanol solvent used, the plant part extracted, and variations in active compound content. Overall, these results confirm previous findings that 70% ethanol extract of cardamom fruit has significant toxic potential and has the potential to be further developed as a source of bioactive compounds, while still considering safety aspects of its use [27].

Toxicity testing on the 70% ethyl acetate extract of cardamom fruit yielded an LC50 value of 181.953 ppm, which, according to Mayer et al.'s classification, falls into the moderately toxic category, with a range of 100-500 ppm. These values indicate that the extract has a fairly strong toxicity against Artemia salina larvae, potentially containing active metabolites that can cause cytotoxic effects. These characteristics are relevant in the context of anticancer agent development, as compounds that exhibit moderate to high toxicity in the BSLT test typically have the ability to inhibit cancer cell growth [28].

The relationship between log concentration and probit values for larval mortality was analyzed using linear regression, yielding the equation y = 3.4936x - 2.8954 with a coefficient of determination R^2 = 0.9796. An R^2 value approaching 1 indicates a very strong relationship between extract concentration and the toxic response of larvae. Based on this regression equation, the LC50 value of the 70% ethyl acetate extract is below 1000 ppm, thus categorizing the extract as toxic.

The toxic properties of the 70% ethyl acetate extract of cardamom fruit are related to the phytochemical test results of the 70% ethyl acetate extract, which showed the detection of steroids. Steroids have biological activity that affects the stability and permeability of cell membranes through interactions with membrane lipid components. Disruption of this membrane structure can cause ion imbalance and disrupt physiological cell function, ultimately leading to the death of *Artemia salina* larvae [28].

Results of GC-MS analysis of 70% ethyl acetate extract of cardamom fruit identified volatile and semi-nonpolar compounds such as eucalyptol, o-xylene, benzene alkyl derivatives, α-terpineol, n-hexadecanoic acid, and several other hydrocarbons. These compounds are known to possess biological activity that can disrupt the nervous system and cellular respiration of test organisms. The presence of terpenoid compounds such as eucalyptol and α-terpineol aligns with the phytochemical test results of the 70% ethyl acetate extract, which showed positive results for steroids, considering that these compounds belong to the lipophilic terpenoid group that is optimally extracted using ethyl acetate solvent [29].

The toxicity observed in the 70% ethyl acetate extract of cardamom fruit is suspected to be due to the contribution of steroid compounds and volatile compounds from the GC-MS analysis. The use of the semi-nonpolar ethyl acetate solvent allows for optimal extraction of lipophilic compounds, resulting in an extract with significant toxic activity. These results indicate that the 70% ethyl acetate extract of cardamom fruit has potential as a source of bioactive compounds. However, further testing is needed to confirm its safety and more in-depth toxicity mechanisms [26].

The toxicity test results of the 70% ethyl acetate extract of cardamom fruit in this study showed an LC50 value <1000 ppm, thus categorizing it as toxic. This finding aligns with several previous studies that

reported that ethyl acetate extracts of plants from the Zingiberaceae family have moderate to strong toxic activity in the Brine Shrimp Lethality Test (BSLT). Previous research has suggested that ethyl acetate extracts tend to extract lipophilic compounds such as steroids and terpenoids, which play a key role in causing toxic effects through disruption of cell membranes and the physiological systems of test organisms. The differences in reported toxicity levels between studies are thought to be influenced by variations in the plant parts used, growing conditions, extraction methods, and the concentration of ethyl acetate solvent. Overall, these results reinforce previous findings that 70% ethyl acetate extract of cardamom fruit has significant toxic potential and indicate the presence of bioactive compounds that require further investigation in terms of both safety and pharmacological potential [27].

CONCLUSION

This study demonstrated that the extraction of cardamom fruit (*Wurfbainia compacta* Sol. ex Maton) using 70% ethanol and 70% ethyl acetate solvents produced yields of 6.98% and 2.80%, respectively. Phytochemical tests showed that the 70% ethanol extract contained flavonoids, tannins, saponins, and steroids, while the 70% ethyl acetate extract contained steroids. GC–MS analysis identified volatile and bioactive compounds that potentially have antioxidant, anti-inflammatory, and cytotoxic activities. The BSLT test showed that the LC50 values of the 70% ethanol and 70% ethyl acetate extracts were 170.506 ppm and 181.95 ppm, respectively, which are classified as moderately toxic. Overall, the 70% ethanol extract of cardamom shows greater potential to be developed as a source of natural anticancer ingredients.

Author Contributions: N. N. Prihantini served as the first and corresponding author and contributed to study conceptualization, experimental design, data interpretation, and manuscript preparation. F. Sitompul contributed to phytochemical analysis, toxicity evaluation, and scientific review. E. G. M. Simanjuntak contributed to laboratory investigation, data verification, and manuscript refinement. B. F. Pangaribuan contributed to experimental support, data documentation, and manuscript editing. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

Declaration of Generative AI and AI-Assisted Technologies: Generative artificial intelligence (AI) tools were used during the preparation of this manuscript solely to assist in the development of the graphical abstract and to improve the English language through proofreading and grammatical correction.

REFERENCES

[1] A. Tarigan and H. Saragih, “Identifikasi kandungan senyawa bioaktif buah kapulaga (*Amomum compactum*),” *J. Gizi*, vol. 12, no. 1, pp. 46–51, 2023, doi:

10.26714/jg.12.1.2023.46-51.

[2] M. Irfan and H. Haryoto, "Aktivitas farmakologi dan kadar senyawa flavonoid total dari tanaman kapulaga (*Amomum compactum*)," *Usadha J. Pharm.*, pp. 205–217, 2022, doi: 10.23917/ujp.v1i2.117.

[3] R. D. Kusumawati, A. Yuniastuti, R. Susanti, and W. H. Nugrahaningsih, "Studi in silico potensi senyawa bioaktif pada kapulaga Jawa (*Amomum compactum*) sebagai antiinflamasi," pp. 304–309, 2021. [Online]. Available: https://proceeding.unnes.ac.id/semnasbiologi/article/view/800/708

[4] R. Fahmida, "Performa ultrasound-assisted extraction senyawa bioaktif buah kapulaga Jawa (*Amomum compactum* Sol. ex Maton) dengan variasi amplitudo dan konsentrasi asam sitrat," Universitas Gadjah Mada, Yogyakarta, Indonesia, 2025. [Online]. Available: https://etd.repository.ugm.ac.id/penelitian/detail/260318

[5] N. Muna, N. Zakiah, V. Aulianshah, M. Munira, and A. Sari, "Efek sitotoksik ekstrak buah kapulaga Jawa (*Amomum compactum* Soland. ex Maton)," *J. SAGO Gizi Kesehat.*, vol. 1, no. 1, pp. 79–84, 2019, doi: 10.30867/gikes.v1i1.302.

[6] R. D. Yudhani, R. N. Pesik, S. Azzahro, A. F. Anisa, and R. Hendriyani, "Acute toxicity test of *Amomum cardamomum* (kapulaga) seed extract on hepatic trasaminase enzyme in Winstar rats," *Indones. J. Clin. Pharm.*, vol. 9, no. 4, 2020, doi: 10.15416/ijcp.2020.9.4.288.

[7] P. Artini, K. W. Astuti, and N. K. Warditiani, "Uji fitokimia ekstrak etil asetat rimpang bangle (*Zingiber purpureum* Roxb.)," *J. Farm. Udayana*, vol. 2, no. 4, 2013. [Online]. Available: https://www.neliti.com/id/publications/279805/uji-fitokimia-ekstrak-etil-asetat-rimpang-bangle-zingiber-purpureum-roxb

[8] A. Nofriyaldi, S. R. Nur Endah, and A. P. Mutiara, "Efektivitas daya antibakteri ekstrak etanol buah kapulaga (*Amomum compactum* Sol. ex Maton) terhadap pertumbuhan *Propionibacterium acnes*," *Media Inf.*, vol. 19, no. 1, pp. 67–74, 2023, doi: 10.37160/bmi.v19i1.123.

[9] P. N. Ravindran and K. J. Madhusoodanan, Eds., *Cardamom: The Genus Elettaria*. CRC Press, 2002.

[10] I. A. Khan and E. A. Abourashed, *Leung's Encyclopedia of Common Natural Ingredients: Used in Food, Drugs and Cosmetics*, 3rd ed. John Wiley & Sons.

[11] A. W. Ningsih *et al*., "Artikel review: Studi fitokimia dan aktivitas farmakologi pada tumbuhan kapulaga (*Elettaria cardamomum* (L.) Maton)," *FARMASIS: J. Sains Farm.*, vol. 4, no. 1, pp. 42–47, 2023. [Online]. Available: https://jurnal.unipasby.ac.id/index.php/farmasis/article/download/6408/4722

[12] S. Sreedharan, V. Nair, and L. Cisneros-Zevallos, "Protective role of phenolic compounds from whole cardamom (*Elettaria cardamomum* (L.) Maton) against LPS-induced inflammation in colon and macrophage cells," *Nutrients*, vol. 15, no. 13, Art. no. 2965, 2023, doi: 10.3390/nu15132965.

[13] W. Nurcholis, D. N. S. Putri, H. Husnawati, S. I. Aisyah, and B. P. Priosoeryanto, "Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of *Amomum compactum* fruits," *Ann. Agric. Sci.*, vol. 66, no. 1, pp. 58–62, 2021, doi: 10.1016/j.aoas.2021.04.001.

[14] R. Kumari, P. K. Chaurasia, S. Yadav, and N. Parween, "Phytochemistry, biological activities, and therapeutic potential of *Elettaria cardamomum* and its medicinal perspectives," in *Exploration of the Medicinal Potential of Kitchen Ingredients*. CRC Press, 2026, pp. 102–114. [Online]. Available: https://www.taylorfrancis.com/chapters/edit/10.1201/9781003559894-10/phytochemistry-biological-activities-therapeutic-potential-elettaria-cardamomum-medicinal-perspectives-rima-kumari-pankaj-kumar-chaurasia-shweta-yadav-nuzhat-parween

[15] M. Al-Owaisi, N. Al-Hadiwi, and S. A. Khan, "GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves," *Asian*

Pac. J. Trop. Biomed., vol. 4, no. 12, pp. 964–970, 2014, doi: 10.12980/APJTB.4.201414B295.

[16] J. Junaidi *et al.*, "GC-MS analysis of bioactive compounds of ethanol extract of soybean *Glycine max* (L.) Merril.," *Trop. J. Nat. Prod. Res.*, vol. 9, no. 4, 2025, doi: 10.26538/tjnpr/v9i4.26.

[17] Abdullah *et al.*, "Recent advances in the extraction, chemical composition, therapeutic potential, and delivery of cardamom phytochemicals," *Front. Nutr.*, vol. 9, Art. no. 1024820, 2022, doi: 10.3389/fnut.2022.1024820.

[18] S. Megawati, M. Safitri, S. N. Rangkuti, L. Makiyyah, and N. I. A. Mawarni, "Test of antibacterial activity and GC-MS analysis of ethyl acetate and *n*-hexane fractions of Kenitu leaves (*Chrysophyllum cainito* L.)," *J. Farm.*, vol. 7, no. 2, pp. 151–161, 2025, doi: 10.35451/jfm.v7i2.2421.

[19] S. F. Sanou, P. Atché, K. Koné, and Y. Soro, "Identification of compounds by GC-MS analysis of the ethyl acetate fraction of the hydroalcoholic extract of *Lippia multiflora* leaves before extraction of the essential oil," *J. Mater. Environ. Sci.*, vol. 15, no. 8, pp. 1150–1173, 2024. [Online]. Available: https://www.jmaterenvironsci.com/Document/vol15/vol15_N8/JMES-2024-1508076-Sanou.pdf

[20] A. U. Gama and A. Tukur, "Phytochemical screening and GC-MS profiling of *Ziziphus jujuba* ethyl acetate leaves extract," *J. Chem. Soc. Niger.*, vol. 50, no. 5, 2025, doi: 10.4314/jcsn.v50i5.1.

[21] P. O. Nyalo, G. I. Omwenga, and M. P. Ngugi, "Antibacterial properties and GC-MS analysis of ethyl acetate extracts of *Xerophyta spekei* (Baker) and *Grewia tembensis* (Fresen)," *Heliyon*, vol. 9, no. 3, 2023, doi: 10.1016/j.heliyon.2023.e14461.

[22] Sufiana and Harlia, "Uji aktivitas antioksidan dan sitotoksisitas campuran ekstrak metanol kayu sepang (*Caesalpinia sappan* L.) dan kulit kayu manis (*Cinnamomum burmannii* B.)," *J. Kim. Khatulistiwa*, vol. 3, no. 2, 2014. [Online]. Available: https://jurnal.untan.ac.id/index.php/jkkmipa/article/download/8428/8435

[23] P. Y. Pratiwi, F. Nurhaeni, A. Fitriasari, and T. Herdyaningtyas, "Perbandingan aktivitas antioksidan ekstrak etanol daun dan buah kapulaga (*Amomum compactum*) dengan metode DPPH (1,1-difenil-2-pikrilhidrazil)," pp. 265–274, 2024. [Online]. Available: https://journal.dea-publishing.id/index.php/prosiding_polkesta/article/download/49/27/83

[24] B. Meyer, N. Ferrigni, J. Putnam, L. Jacobsen, D. Nichols, and J. McLaughlin, "Brine shrimp: A convenient general bioassay for active plant constituents," *Planta Med.*, vol. 45, no. 5, pp. 31–34, 1982, doi: 10.1055/s-2007-971236.

[25] A. Surya, R. Murwindra, and M. S. Fiki, "Uji toksisitas ekstrak etanol daun kemangi (*Ocinum sanctum* L.) terhadap larva udang (*Artemia salina* L.) dengan metode BSLT (Brine Shrimp Lethality Test)," *JEDCHEM (J. Educ. Chem.)*, vol. 4, no. 1, pp. 5–7, 2022, doi: 10.36378/jedchem.v4i1.1886.

[26] D. M. Ulfa, S. M. Tulandi, and J. Sulistiyo, "Toxicity evaluation with Brine Shrimp Lethality Test and phytochemical analysis of some Indonesian plant extracts as potential anti-colon cancer agents," *Trop. J. Nat. Prod. Res.*, vol. 8, no. 4, pp. 6864–6867, 2024, doi: 10.26538/tjnpr/v8i4.16.

[27] A. R. Bareta, E. L. Widiastuti, and N. Nurcahyani, "Uji sitotoksisitas taurin dan ekstrak etanol makroalga cokelat dengan metode BSLT (Brine Shrimp Lethality Test)," *Ber. Biol.*, vol. 22, no. 2, pp. 153–157, 2023, doi: 10.55981/beritabiologi.2023.660.

[28] A. Hermawanfutri and S. Hazar, "Uji sitotoksik ekstrak etanol kulit batang awar-awar dengan metode BSLT," *J. Ris. Farm.*, pp. 81–88, 2023, doi: 10.29313/jrf.v3i2.3117.

[29] D. Sukandar, S. Hermanto, and E. Lestari, "Uji toksisitas ekstrak daun pandan wangi (*Pandanus amaryllifolius* Roxb.) dengan metode Brine Shrimp Lethality Test (BSLT)," *J. Kim. Val.*, 2008, doi: 10.15408/jkv.v1i2.217