Advances in Pharmacology and Pharmacy 13(4A): 747-758, 2025 http://www.hrpub.org
DOI: 10.13189/app.2025.130701

Phytochemical Investigation and Evaluation of Antioxidant, Antimicrobial, and Anticancer Activities of *Elettaria cardamomum* Pod Extracts

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Received July 31, 2024; Revised February 25, 2025; Accepted September 21, 2025

Cite This Paper in the Following Citation Styles

(a): *[1] Sowmya KM, Narendhirakannan RT , "Phytochemical Investigation and Evaluation of Antioxidant, Antimicrobial, and Anticancer Activities of Elettaria cardamomum Pod Extracts," Advances in Pharmacology and Pharmacy, Vol. 13, No. 4A, pp. 747 - 758, 2025. DOI: 10.13189/app.2025.130701.*

(b): *Sowmya KM, Narendhirakannan RT (2025). Phytochemical Investigation and Evaluation of Antioxidant, Antimicrobial, and Anticancer Activities of Elettaria cardamomum Pod Extracts. Advances in Pharmacology and Pharmacy, 13(4A), 747 - 758. DOI: 10.13189/app.2025.130701.*

Abstract This study investigates the phytochemical composition, antimicrobial, antioxidant, and anticancer activities of the aqueous, chloroform, and ethanol extracts of *Elettaria cardamomum* pods. Phytochemical screening revealed the presence of alkaloids and flavonoids in the aqueous extract, cardiac glycosides and alkaloids in the ethanol extract, and only cardiac glycosides in the chloroform extract, with tannins, terpenoids, and saponins absent across all extracts. Focusing on flavonoids, the total flavonoid content of the aqueous extract was quantified at 45.5 ± 0.12 µg/mL using the aluminum chloride method. Antimicrobial assays demonstrated that the aqueous extract inhibited both Gram-positive and Gram-negative bacteria, with the highest inhibitory effect observed against *Pseudomonas aeruginosa* (18±0.02 mm zone of inhibition at 100 µg/mL). The extract also exhibited significant antioxidant properties, displaying effective DPPH scavenging (IC_{50} = 20.44 ± 0.23 µg/mL), ABTS radical inhibition (IC_{50} = 10.324±0.23 µg/mL), and nitric oxide scavenging (IC_{50} = 21.78±0.18 µg/mL). Additionally, the anticancer potential was assessed via MTT assay, showing significant growth inhibition of the HT-29 colorectal cancer cell line (IC_{50} = 67.98 µg/mL), with maximum inhibition at 300 µg/mL (71.93%). The findings of this study highlight the therapeutic potential of *E. cardamomum* pods as a source of bioactive compounds with notable antimicrobial, antioxidant, and anticancer activities. These results support further exploration of *E. cardamomum* in medicinal applications, emphasizing its role as a valuable natural product in combating oxidative stress and cancer. Future research should aim to isolate and characterize the specific compounds responsible for these bioactivities to develop effective therapeutic agents.

Keywords Anticancer, Antimicrobial, Antioxidant, Colon Cancer, *Elettaria cardamomum*

1. Introduction

Colorectal cancer, commonly referred to as colon cancer, poses a significant global health threat, affecting millions of individuals annually and ranking among the leading causes of cancer-related deaths worldwide. Despite recent advancements in medical therapies, the need for more effective and accessible treatments remains critical, as current interventions often come with substantial morbidity and can significantly impair patients' quality of life [1]. Standard treatments, including radiation therapy, chemotherapy, and surgery, can result in a range of adverse effects, both physical and psychological, highlighting the urgent need for alternative therapeutic strategies [2]. In recent years, there has been a growing interest in exploring natural sources and alternative therapies for colorectal cancer. Medicinal plants, with their diverse chemical

compositions and biological activities, present promising candidates for the development of new therapeutic agents [3]. Many herbs and spices have been historically utilized for their health benefits, and emerging research suggests that they may possess bioactive components capable of preventing and treating cancer. These natural products not only add flavor to our diets but also offer potential protective effects against cancer and other chronic diseases [4].

The role of spices in drug discovery is increasingly recognized for its potential to uncover novel therapeutic agents and enhance existing treatments. Historically, many spices have been integral to traditional medicine systems, where they were used for their healing properties long before modern pharmacology emerged [5]. These are rich in bioactive compounds such as alkaloids, flavonoids, and terpenoids, and these culinary ingredients offer a diverse array of pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial effects [6]. Their unique chemical structures can serve as inspiration for new drug development, while their synergistic properties may improve the efficacy of conventional medications [7]. Furthermore, integrating traditional knowledge about spices into contemporary research not only enriches the drug discovery process but also ensures that new therapies resonate culturally and are more readily accepted. As the quest for effective and sustainable treatments continues, the exploration of spices represents a promising frontier in the field of pharmacology [8].

Elettaria cardamomum (L.) Maton, commonly known as cardamom, is cultivated in regions such as India, Guatemala, Sri Lanka, Nepal, Indonesia, Costa Rica, Mexico, and Tanzania. Cardamom has long been used to treat allergies, nausea, diarrhea, renal diseases, digestive issues, and gum infections [9]. This spice has garnered attention for its anti-inflammatory properties, which may help alleviate digestive disorders and reduce intestinal inflammation [10]. The phytochemical composition of cardamom seeds includes proteins, sterols, terpenoids, flavonoids, starch, and tannins, all of which contribute to its therapeutic potential [11]. Research indicates that the anticancer effects of cardamom are mediated through various mechanisms, including anti-inflammatory, anti-proliferative, pro-apoptotic, and antioxidant activities [12]. Recent studies have indicated that the bioactive compounds found in cardamom play a critical role in modulating signaling pathways associated with cancer progression and metastasis [13]. These compounds have been shown to suppress inflammation and oxidative stress, both of which are implicated in the development of cancer [14]. Additionally, cardamom's flavonoids have been linked to enhanced immune responses, which could further aid in combating cancer growth [15]. Studies have demonstrated that *E. cardamomum* can inhibit the growth of various cancer cell types, including skin, lung, myeloma, and oral squamous cell carcinoma, while also inducing apoptosis in these cells. Furthermore, it shows promise in targeting cells that develop resistance to chemotherapy, particularly in breast and colon cancers [16].

Given the accessibility and affordability of cardamom pods compared to seeds, our study aims to investigate the anticancer potential of *E. cardamomum* pods specifically against colorectal cancer. To the best of our knowledge, this research represents a pioneering effort to evaluate the anticancer activity of *E. cardamomum* pods. In this study, we conducted a comprehensive phytochemical analysis and assessed the in vitro antioxidant, antimicrobial, and anticancer activities of the *E. cardamomum* extract against the human colorectal cancer cell line (HT-29). By elucidating the potential health benefits of cardamom pods, we hope to contribute valuable insights into natural therapeutic alternatives for colorectal cancer treatment, potentially paving the way for future clinical applications and more holistic approaches to cancer management.

2. Materials and Methods

2.1. Acquisition of Plant Material and Extraction

The plant *Elettaria cardamomum* (L.) Maton was collected from Mananthavady, Wayanad District, Kerala, India. The identification and authentication of the plant were performed at the Department of Botany at Kongunadu Arts and Science College, Coimbatore. The pods were carefully removed, washed with sterile water to eliminate any contaminants, and then shade-dried at room temperature until they reached a constant weight. The dried pods were ground into a fine powder using a clean electric grinder.

For extraction, 50 g of the powdered sample was dissolved in 500 mL of respective solvents, including sterile distilled water, chloroform, and ethanol. The mixtures were heated on a magnetic stirrer for optimal extraction. After stirring, the extracts were filtered through Whatman No. 1 filter paper to separate the solid residues. The filtered extracts were collected in sterile containers and stored at 4 ℃ for further analysis.

2.2. Preliminary Phytochemical Investigation

The phytochemical composition of the various extracts of *E. cardamomum* pods was assessed to identify the presence of key bioactive compounds, including tannins, terpenoids, saponins, cardiac glycosides, flavonoids, and alkaloids. Standard biochemical procedures, as outlined in the review by Velavan [17], were employed for this investigation.

2.3. Determination of Total Flavonoid Content

The total flavonoid content in the aqueous extract was quantified using the aluminum chloride colorimetric method. Different concentrations of the extract (ranging

from 25 to 1000 µg/mL) were combined with 2.8 mL of distilled water, 0.1 mL of 1% sodium acetate, and 0.1 mL of 10% aluminum chloride. The reaction mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 415 nm using a UV-Vis spectrophotometer. Quercetin was utilized as the standard reference for constructing a standard curve, from which the flavonoid content was expressed as mg/dL [18].

2.4. Antimicrobial Activity

2.4.1. Agar Well Diffusion Method

The antimicrobial efficacy of the aqueous extract of *E. cardamomum* pods was evaluated against clinical isolates of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, and Pseudomonas sp.*, which were obtained from the Department of Biochemistry at Kongunadu Arts and Science College, Coimbatore. Bacterial cultures were maintained on nutrient broth at 37 ℃. Using a sterile cork borer (6 mm diameter), wells were created in nutrient agar plates that had been inoculated with overnight cultures of each test organism. Different concentrations of the test extract (25, 50, 75, 100 µg/mL) were added to the wells, which were then incubated at 37 ℃ for 24 hours. The zones of inhibition around each well were measured post-incubation to assess antimicrobial activity [19].

2.5. Antioxidant Activity

2.5.1. (DPPH) -1, 1-Diphenyl-2-picrylhydrazyl Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the aqueous extract was measured following the method reported by Amalraj et al. [20]. Various concentrations (25, 50, 75, 100, 250, 500, 750, and 1000 µg/mL) of the extract were mixed with 3 mL of freshly prepared 0.1 mM methanolic DPPH solution. The reaction mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer.

2.5.2. ABTS Radical Decolorization Assay

The antioxidant potential of the aqueous extract was assessed using the ABTS radical decolorization assay, as described by Thangaraj [21]. A 7 mM ABTS solution was mixed with 88 mL of 140 mM potassium persulfate and allowed to incubate in the dark at room temperature for 14 hours. The ABTS+ solution was diluted with ethanol to achieve an optical density of 0.700 ± 0.02 at 734 nm. Different concentrations of the aqueous extract (25, 50, 75, 100, 250 µg/mL) were treated with 2 mL of the ABTS+ solution and incubated at 30 ℃ for 10 minutes. The absorbance was recorded at 734 nm.

% of radical scavenging activity = (A control A test)/A control) X 100

2.5.3. Nitric Oxide Scavenging Assay

The nitric oxide scavenging activity of the aqueous extract was determined by mixing various aliquots of the extract with 3 mL of 10 mM sodium nitroprusside and incubating the mixture at room temperature for 150 minutes. Following incubation, 3 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The optical density was measured at 546 nm using a spectrophotometer [21].

% of radical scavenging activity = (A control – A test)/A control) X 100

2.6. Anticancer Activity

The anti-cancer activity of the *E. cardamomum* pod extract was assessed using the MTT assay. The Human colorectal cancer cell line (HT-29) was procured from the Biogenix Research Center for Molecular Biology and Applied Science in Bangalore, India. The cell line was cultured in Minimum Essential Medium (MEM) supplemented with fetal bovine serum and incubated in a 5% CO_2 atmosphere at 37 ℃. After 24 hours, the growth medium was replaced with fresh medium containing various aliquots of the extract, and the cells were incubated for an additional 24 hours.

Post-incubation, the morphology of the cells was observed using an inverted phase contrast microscope, and any cytotoxic effects were documented through photographic evidence. Subsequently, 10 µL of MTT solution was added to each well. Following the removal of the supernatant, the insoluble formazan crystals were dissolved in 200 µL of DMSO (MTT solubilization solution). The absorbance was measured at 570 nm using a microplate reader [22].

The percentages of viability (%V) and inhibition (%I) were calculated as:

$$\text{Percentage of viability (\%V)} = 100(A_t / A_c)$$

$$\text{Percentage of inhibition (\%I)} = 100\,[1 - (A_t / A_c)]$$

Where, A_t – absorbance of treated cells; A_c - absorbance of control cells.

2.7. Statistical Analysis

The experiments were carried out in triplicate. Data are presented as mean ± standard deviation. Significant differences between groups were evaluated using the ANOVA. p-values less than 0.05 were considered significant.

3. Results

3.1. Preliminary Phytochemical Investigation

The aqueous, chloroform, and ethanol extract of *E.*

cardamomum pod was screened for the presence of various phytochemicals using standard biochemical procedures. Alkaloids and flavonoids exist in the aqueous extract of *E. cardamomum* pod, whereas the ethanol extract contains cardiac glycosides and alkaloids. The chloroform extract showed positive results only for cardiac glycosides. The phytochemicals such as tannins, terpenoids, and saponins were absent in all three extracts (Aqueous, ethanol, and chloroform). The results of the preliminary phytochemical investigation are provided in **Table 1**. As our study focused on flavonoids, further studies have proceeded with the aqueous extract of *E. cardamomum* pod as it only showed positive results for flavonoids.

3.2. Determination of Total Flavonoid Content

In the present study, the total flavonoid content of the aqueous extract of the *E. cardamomum* pod was evaluated by the Aluminium chloride method using quercetin as the standard compound **(Table 2).** The flavonoid content present in the aqueous extract of *E. cardamomum* was found to be **45.5 ± 0.12** µg/m. **Figure 1** shows the flavonoid content present in each concentration of *E. cardamomum* pod.

Table 1. Phytochemical investigation of *Elettaria cardamomum* pod extract

Phytochemical	Tests	Aqueous	Ethanol	Chloroform
Tannins	Ferric chloride test	-	-	-
Terpenoid	Salkowaski test	-	-	-
Saponin	Foam test	-	-	-
Cardiac glycosides	Keller-Killani test	-	+	+
Flavonoid	Ammonium Test	+	-	-
	Aluminium chloride test	+	-	-
Alkaloid	Wagner's test	+	+	-
	Meyer's test	+	+	-

+ - presence ; -- - absence

Table 2. Total flavonoid content of *E. cardamomum* Pod

Concentration (µg/ml)	FLAVONOID	
	QUERCETIN (STD)	POD (mg/g of aqueous extract)
20	0.448 ±0.06	2.06 ±0.04
40	0.617 ±0.03	2.63 ±0.19
60	0.791 ±0.12	3.09 ±0.01
80	0.957 ±0.15	4.32 ±0.08
100	0.292 ±0.11	4.55 ±0.12

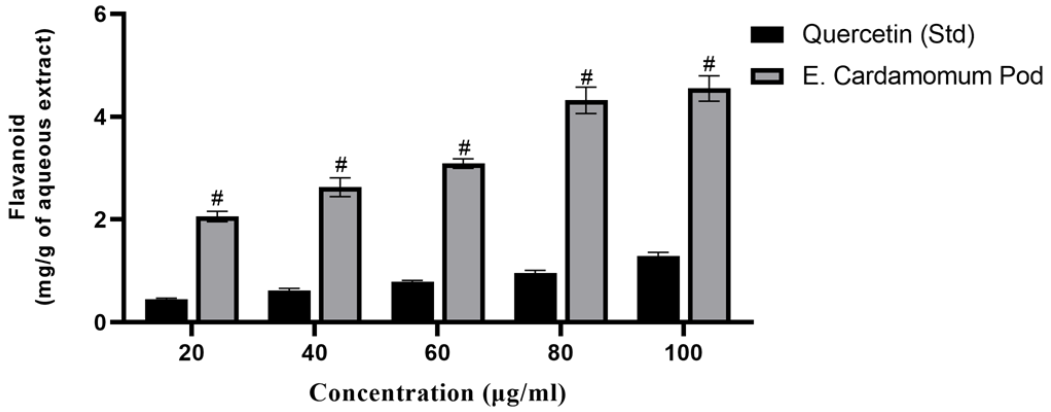


Figure 1. Total flavonoid content in aqueous extract of E.cardamomum Pod. The graph represents the mean ±SD of the total flavonoid content of different concentrations of aqueous extract of E.Cardamomum Pod and the standard quercetin. # represents the statistical significance of less than 0.005 comparing the standard quercetin and E.Cardamomum Pod for each concentration using a simple t-test

3.3. Antimicrobial Activity

The results of the antimicrobial activity of *E. cardamomum (L) maton* pod are provided in **Table 3.** Aqueous extract of *E. cardamomum* pod was examined against four test organisms (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa*). Both the Gram-positive and Gram-negative bacteria strains were sensitive to the extracts at concentrations ranging from 25 to 100 µg. The highest inhibitory zone was seen against *Pseudomonas aeruginosa* (18±0.02mm), followed by *Escherichia coli* (16±0.08mm), and *Klebsiella pneumoniae* (16±0.04mm) at a concentration of 100µg/ml. The minimum zone of inhibition was observed for *Staphylococcus aureus* (14±0.13mm) (**Figure 2)**.

Based on standard interpretive criteria, an inhibition zone greater than 15 mm is typically indicative of susceptibility, suggesting that *E. cardamomum* extracts may be effective against these pathogens. These findings highlight the potential of *E. cardamomum* as a source of antimicrobial agents, warranting further investigation into its clinical applications.

3.4. Antioxidant Activity

3.4.1. (DPPH) -1, 1-Diphenyl-2-picrylhydrazyl Radical Scavenging Activity

The *E. cardamomum* pod extract demonstrated effective DPPH free radical scavenging activity in a concentration-dependent manner, as shown in **Table 4**. The EC_{50} for *E. cardamomum* pod extract was 206.6 µg/ml (log EC_{50} = 2.315), with a 95% confidence interval ranging from 180.5 to 236.6 µg/ml (log EC_{50}: 2.257 to 2.374). In comparison, the ascorbic acid standard exhibited an EC_{50} of 139.0 µg/ml (log EC_{50} = 2.143) with a confidence interval from 131.4 to 147.0 µg/ml (logEC_{50}: 2.119 to 2.167). The maximum DPPH radical scavenging activity of the *E. cardamomum* pod extract was achieved at a concentration of 1000 µg/ml (80.07 ± 0.19%), while the lowest scavenging activity was observed at 25 µg/ml, with 10.43 ± 0.26% inhibition. The concentration-dependent DPPH radical scavenging activity of the *E. cardamomum* pod extract is illustrated in **Figure 3.**

Table 3. Antimicrobial activity of *E. cardamomum* pod

S. No.	Test organisms	Zone of inhibition (mm)			
		25µg	**50 µg**	**75 µg**	**100 µg**
1	*Staphylococcus aureus*	8±0.01	10±0.12	12±0.15	14±0.13
2	*Escherichia coli*	8±0.04	12±0.13	14±0.12	16±0.08
3	*Klebsiella pneumoniae*	8±0.02	14±0.10	14±0.09	16±0.04
4	*Pseudomonas aeruginosa*	6±0.03	12±0.10	13±0.13	18±0.02

Values = Mean ± Standard deviation

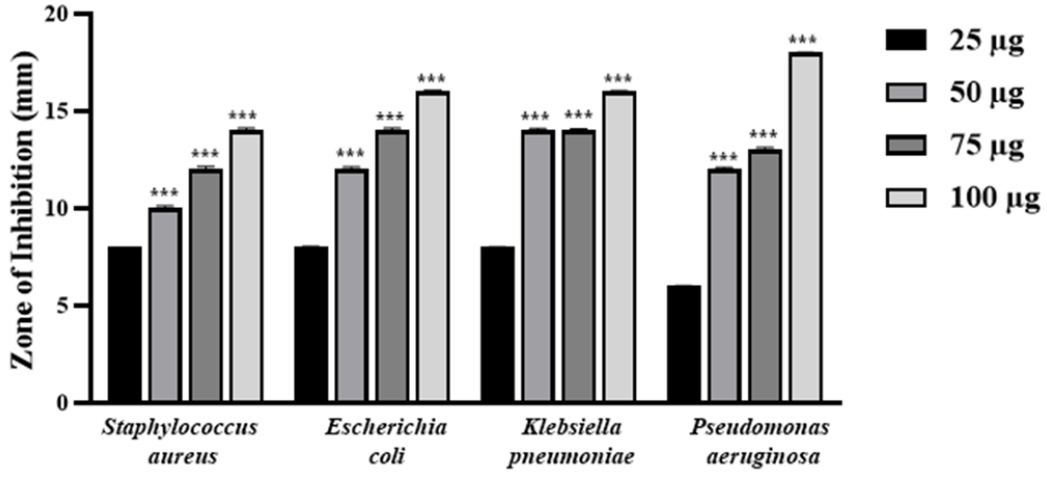


Figure 2. Antimicrobial activity of *E. cardamomum* pod. The data represents the zone of inhibition (mm) exhibited by different concentrations of *E. cardamomum* pod aqueous extract against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa.* Dunnett's multiple comparison tests the effect of concentration on each bacteria individually and includes the significance as *** which represents the $p<0.005$

Table 4. DPPH) -1, 1-Diphenyl-2-picrylhydrazyl radical scavenging activity of ***E. cardamomum*** **Pod**

Concentration (µg/ml)	**Ascorbic acid (Std)**	***E. cardamomum*** **Pod**
25	12.64 ± 0.05	10.43 ± 0.26
50	27.84 ± 0.21	22.7 ± 0.24
75	38.19 ± 0.23	33.12 ± 0.13
100	44.06 ± 0.14	41.09 ± 0.24
250	62.52 ± 0.13	52.57 ± 0.12
500	75.43 ± 0.16	63.74 ± 0.11
750	81.25 ± 0.22	71.25 ± 0.21
1000	88.73 ± 0.27	80.07 ± 0.19

Values = Mean ± Standard deviation

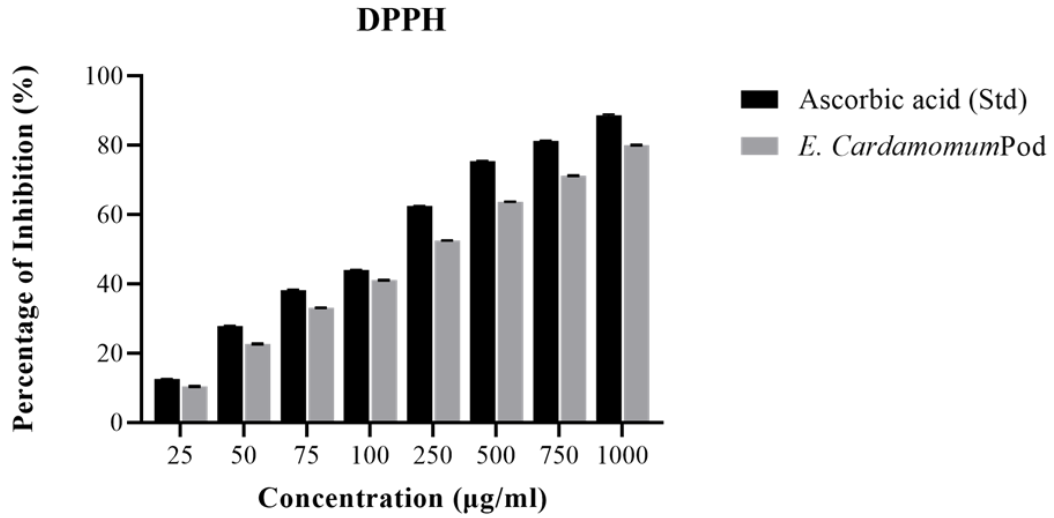


Figure 3. DPPH radical scavenging activity The graph represents the concentration-dependent percentage of inhibition of DPPH scavenging exhibited by *E. cardamomum* pod aqueous extract with an IC_{50} value of 206.6 µg/ml and standard ascorbic acid with an IC_{50} value of 2.143 µg/ml

Table 5. ABTS radical decolourization assay of *E. cardamomum* Pod

Concentration (µg/ml)	**Ascorbic acid (Std)**	***E. cardamomum*** **Pod**
25	10.47 ±0.23	15.7 ± 0.21
50	23.12 ±0.11	23.87 ±0.24
75	34.74 ±0.18	39.56 ±0.15
100	40.35 ±0.13	46.86 ±0.19
250	53.86 ±0.19	58.98 ±0.22
500	65.46 ±0.22	64.39 ±0.10
750	77.32 ±0.17	72.96 ±0.17
1000	86.28 ±0.20	80.45 ±0.14

Values = Mean ± Standard deviation

3.4.2. ABTS Radical Decolorization Assay

The ABTS radical cation scavenging activity of *E. cardamomum* pod extract was found to be concentration-dependent, as presented in **Table 5**. The EC_{50} for the *E. cardamomum* pod extract was 164.8 µg/ml (log EC_{50} = 2.217), with a 95% confidence interval ranging from 166.7 to 205.5 µg/ml (log EC_{50}: 2.222 to 2.313). In contrast, the ascorbic acid standard exhibited an EC_{50} of 2.217 µg/ml (log EC_{50} = 2.267), with a confidence interval from 2.141 to 2.293 µg/ml (log EC_{50}: 2.222 to 2.313). The

highest ABTS radical scavenging activity of *E. cardamomum* pod extract was observed at a concentration of 1000 µg/ml, achieving 80.45 ± 0.14% inhibition, while the lowest activity occurred at 25 µg/ml, with 15.7 ± 0.21% inhibition. The percentage of inhibition of *E. cardamomum* pod extract against the ABTS cation is illustrated in **Figure 4**.

3.4.3. Nitric Oxide Scavenging Assay

The NO radical cation scavenging activity of *E. cardamomum* pod extract was found to be concentration-dependent, as presented in **Table 6**. The EC_{50} for the *E. cardamomum* pod extract was 157.0 µg/ml (log EC_{50} = 114.5), with a 95% confidence interval ranging from 128.0 to 192.7 µg/ml (log EC_{50}: 94.93 to 138.1). In contrast, the ascorbic acid standard exhibited an EC_{50} of 2.196 µg/ml (log EC_{50} = 2.059), with a confidence interval from 2.107 to 2.285 µg/ml (log EC_{50}: 1.977 to 2.140). The highest NO radical scavenging activity of *E. cardamomum* pod extract was observed at a concentration of 1000 µg/ml. The percentage of inhibition of *E. cardamomum* pod extract against the ABTS cation is illustrated in **Figure 5**.

3.5. Anti-Cancer Activity

The anticancer efficacy of *E. cardamomum* pod was evaluated through an MTT assay, with results summarized in **Table 3**. After 24 hours, *E. cardamomum* pod demonstrated significant inhibition of HT-29 (human colorectal cancer) cell growth, as detailed in **Table 7**. The pod exhibited an IC_{50} of 86.26 µg/ml, with a log EC_{50} of 1.936. The 95% confidence interval for the IC_{50} ranged from 75.42 to 98.73 µg/ml (log EC_{50}: 1.877 to 1.994), indicating a potent anticancer response. The maximum observed inhibition of cell growth was 71.93% at 300 µg/ml, while the minimum inhibition of 14.62% was noted at 18.75 µg/ml. The concentration-dependent inhibition of cell growth by *E. cardamomum* pod is illustrated in **Figure 6a and b**.

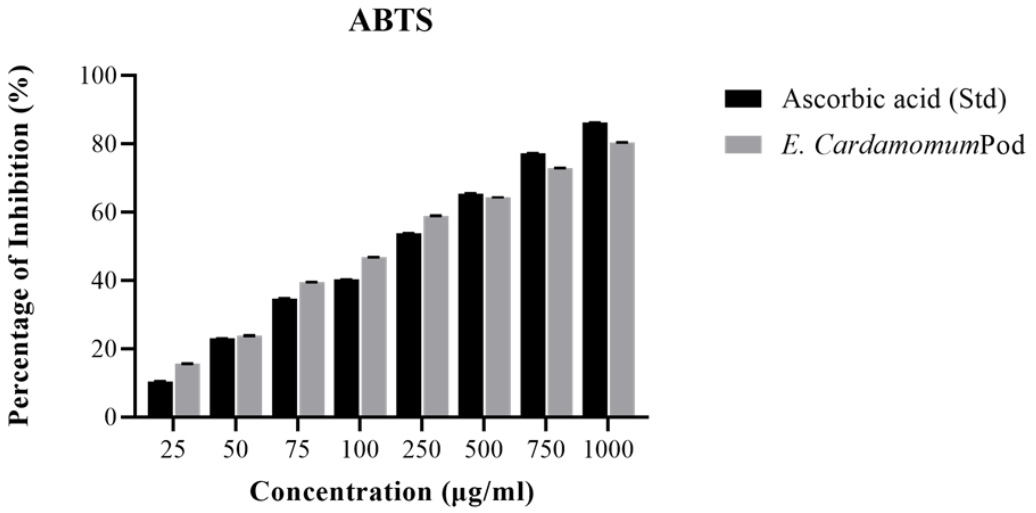


Figure 4. ABTS radical cation decolourization assay The graph represents the concentration-dependent percentage of inhibition of ABTS scavenging exhibited by *E. cardamomum* pod aqueous extract with an IC_{50} value of 164.8 µg/ml and standard ascorbic acid with an IC_{50} value of 2.267 µg/ml

Table 6. Nitric oxide scavenging assay of *E. cardamomum* Pod

Concentration (µg/ml)	Ascorbic acid (Std)	*E. cardamomum* Pod
25	23.78 ± 0.23	23.87 ± 0.10
50	32.65 ± 0.12	30.45 ± 0.05
75	45.39 ± 0.14	38.82 ±0.12
100	51.27 ± 0.25	46.12 ± 0.23
250	61.46 ± 0.16	53.98 ± 0.17
500	66.91 ± 0.11	65.18 ±0.15
750	79.47 ±0.12	73.63 ±0.18
1000	97.13 ±0.09	82.67 ±0.25

Values = Mean ± Standard deviation

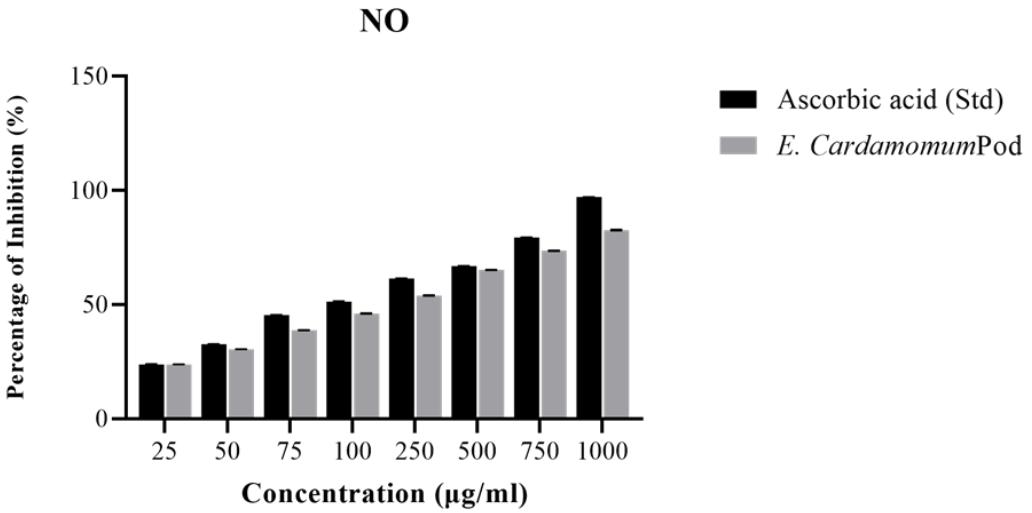
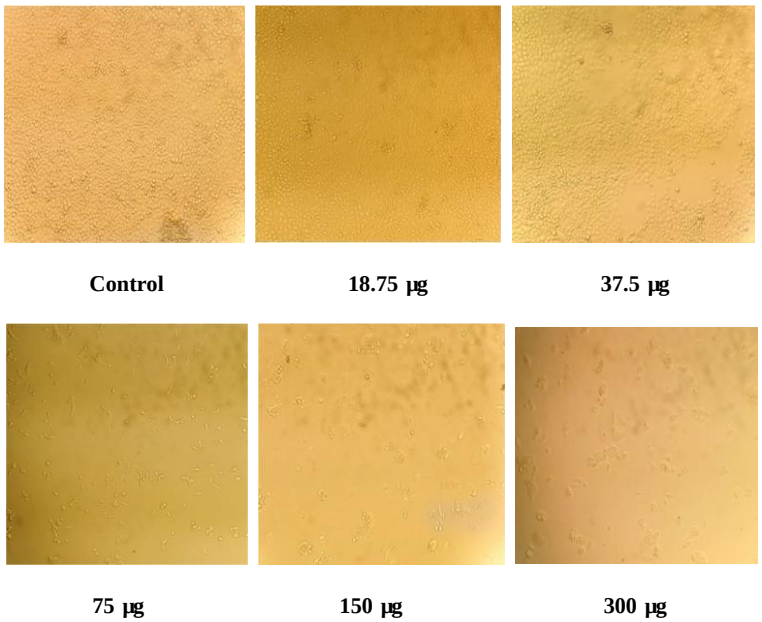


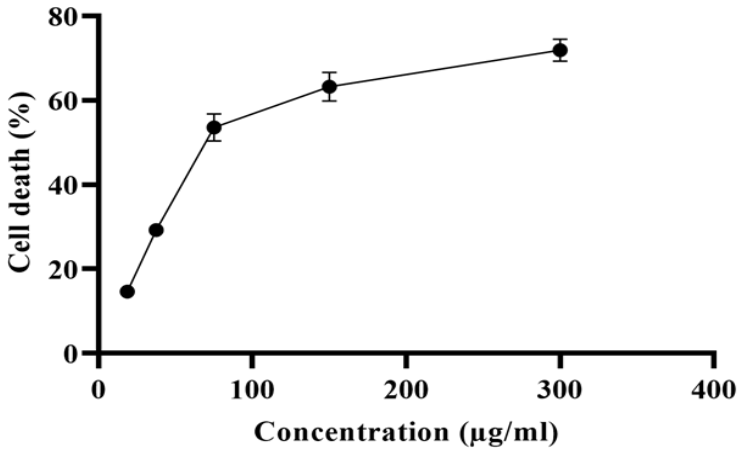
Figure 5. Nitric oxide scavenging assay. The graph represents the concentration-dependent percentage of inhibition of Nitric oxide scavenging exhibited by *E. cardamomum* pod aqueous extract with an IC_{50} value of 157 µg/ml and standard ascorbic acid with an IC_{50} value of 114.5 µg/ml

Table 7. Anti-cancer activity of *E. cardamomum* pod against the HT-29 cell line

Concentration (µg/ml)	Cell inhibition (%)	Anti-cancerous activity
18.75	14.62	Mild
37.5	29.24	Moderate
75	53.55	Severe
150	63.24	Severe
300	71.93	Severe
IC_{50}	**67.98 µg/ml**	



a)



b)

Figure 6. Anti-cancer activity of *E. cardamomum* pod against the HT-29 cell line. (a) depicts a decline in the cell proliferation of HT-29 cells in the presence of increasing concentrations of *E. cardamomum* pod aqueous extract and (b) represents the dose-response curves of MTT assay graphs of *E. cardamomum* pod aqueous extract with an IC_{50} value of 86.26

4. Discussion

Natural products or their derivatives are used in cancer therapy because they not only cause cell apoptosis but also work by regulating cell apoptosis or combination therapy to prevent cancer cells from becoming resistant to chemotherapeutic medications [23]. *E. cardamomum* is widely recognized for its aromatic components and extracted bioactive compounds from dehydrated ripe fruits (seeds and pods), which hold significant value in the food, cosmetic, and pharmaceutical sectors [24]. Traditionally, cardamom has been used to cure a variety of conditions, including renal, intestinal, heart, gum infections asthma, and cataracts. Moreover, it contains anti-inflammatory, antioxidant, hypoglycemic, and anticancer properties [25]. Therefore, we have investigated the potency of *E. cardamomum* pod against the HT-29 cell line as a possible source for treating colorectal cancer.

The phytochemical attributes of medicinal plants were examined to identify and isolate the lead compounds for new drugs from the plants. The characteristics of the phytochemicals in the plants allow one to identify their distinct biological activity [26]. The methanol extract of, according to Ahmed *et al.* [27], the acetone, aqueous, ethanol, and ethyl acetate fraction of the *E. cardamomum* contains phytochemicals such as flavonoids, alkaloids, saponins, and tannins. Likewise, large cardamom fruit (*Amomum subulatum*) is enriched with bioactive components such as phenol and flavonoids necessary for medicinal properties according to the preliminary assessment [28]. Numerous flavonoid components, including luteolin, quercetin, kaempferol, and pelargonidin, are present in the organic fractions of crude cardamom extracts. The high antioxidant content and therapeutic advantages of cardamom spice are attributed to its flavonoids and it can function through a variety of pathways [29].

The ethanolic extract of *E. cardamomum* was tested against bacterial strains such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* [30]. *E. cardamomum* ethanolic extract exhibited a broad-spectrum antibacterial action, and inhibitory zones ranging in diameter from 8 to 33 mm, thereby considerably inhibiting the growth of both gram-positive and gram-negative strains. In our study, we observed inhibition zones for *E. cardamomum* extracts ranging from 14 mm to 18 mm, indicating significant antibacterial activity against the tested strains. Previous studies have reported broader inhibition zones for the ethanolic extract of *E. cardamomum*, ranging from 8 mm to 33 mm, which may reflect variations in extraction methods or bacterial strains used. Consequently, this extract demonstrated dose-dependent antibacterial efficacy against the tested microbial strains [6]. Similarly, the anti-bacterial activity of *E. cardamomum* pods was screened against the multidrug-resistant *Klebsiella pneumonia*, and *Staphylococcus aureus* [31].

E. cardamomum was tested for its ability to donate hydrogen or electrons to convert DPPH to DPPH-H using spectroscopy. Antioxidant activity measurements revealed that raising the concentration of cardamom essential oil decreased the color as well as absorbance of the DPPH solution at 517nm [32]. The ABTS test produces the ABTS radical cation ($ABTS^+$) by the reaction of ABTS with potassium persulfate, which results in the observation of a blue-green color. The radical returns to a colorless state in the presence of the antioxidant [33]. The antioxidant potential of the essential oil of *E. cardamomum* was also demonstrated by Castillo *et al.* and the maximum activity recorded was 79.48% [34]. The radical scavenging properties of *Amomum compactum* Soland Ex Maton Fruit extracts were assessed using ABTS and DPPH assays. The findings revealed the stronger antioxidant activity were

detected when assessed by the ABTS in contrast to the DPPH test [9].

The acetone extract derived from A. compactum exhibited anticancer breast action, with an IC_{50} value of 44.7828 µg/mL [35]. Cardamom extract have cytotoxic effects on the Head and Neck Squamous Cell Carcinoma cell line in a dose- and time-dependent manner. Cardamom extract reduced the percentage of active and proliferating cells upon increasing concentration. However, it enhanced the number of apoptotic cells as well as the amounts of caspase-3 and BAX protein [36]. The zinc oxide nanopartoicles synthesized from *Elettaria cardamomum* (green cardamom) exhibited anti-cancer action against HepG2 cell lines [37].

5. Conclusions

The present study explored the aqueous extract of *E. cardamomum* pod has provided valuable insights into its phytochemical composition, *In-vitro* antioxidant properties, antimicrobial effects, and anticancer activity against colon cancer cells. The study revealed that the extract is rich in various phytochemicals such as flavonoids and alkaloids which contribute to its antioxidant potential. The total flavonoid content of the aqueous extract was 45.5 µg/mL, highlighting its potential health benefits. Furthermore, the aqueous extract showed substantial antimicrobial action against various strains of bacteria indicating its potential as an alternative therapeutic agent against microbial infections. Antimicrobial assays showed effective inhibition of both Gram-positive and Gram-negative bacteria, with an 18 mm zone of inhibition against *Pseudomonas aeruginosa* at 100 µg/mL. The *In-vitro* antioxidant assays demonstrated the extract's potency to quench free radicals and prevent oxidative damage, suggesting its potential as a natural source of antioxidants. The extract also exhibited significant antioxidant activity (IC_{50} values of 20.44 ± 0.23 µg/mL for DPPH, 10.324 ± 0.23 µg/mL for ABTS, and 21.78 ± 0.18 µg/mL for nitric oxide scavenging). Most importantly, the extract showed promising anticancer activity against colon cancer cells, as evidenced by its ability to inhibit cell proliferation and induce cell death. Additionally, it demonstrated notable anticancer activity against the HT-29 colorectal cancer cell line, with an IC_{50} of 67.98 µg/mL and a maximum inhibition of 71.93% at 300 µg/mL. However, further studies, particularly synthesis of nanoparticles using *E. cardamomum* pod extract, *In-vitro*, and *in-vivo* studies are necessary to confirm its efficacy and establish its safety for human consumption or medical use.

Acknowledgements

The authors are grateful to the management as well as the faculty members in the Department of Biochemistry, Kongunadu Arts & Science College (Autonomous), G.N. Mills P.O, Coimbatore, Tamil Nadu-641029, India for providing the support to fulfill this work.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Statement

This work received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author's Contribution

Both authors contributed equally to concept making, data acquiring, investigating, and writing the manuscript.

REFERENCES

[1] Schmoll, H.J., Van Cutsem, E., Stein, A.E.S.M.O., Valentini, V., Glimelius, B., Haustermans, K., Nordlinger, B., Van de Velde, C.J., Balmana, J., Regula, J., Nagtegaal, I.D., "ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making", Annals of Oncology, vol. 23, no. 10, pp. 2479-2516, 2012. https://doi.org/10.1093/annonc/mds236

[2] Ratjen, I., Schafmayer, C., Enderle, J., Di Giuseppe, R., Waniek, S., Koch, M., Burmeister, G., Nöthlings, U., Hampe, J., Schlesinger, S., Lieb, W., "Health-related quality of life in long-term survivors of colorectal cancer and its association with all-cause mortality: a German cohort study", BMC Cancer, vol. 18, pp. 1-15, 2018. https://doi.org/10.1186/s12885-018-5075-1

[3] Dehelean, C.A., Marcovici, I., Soica, C., Mioc, M., Coricovac, D., Iurciuc, S., Cretu, O.M., Pinzaru, I., "Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy", Molecules, vol. 26, no. 4, p. 1109, 2021. https://doi.org/10.3390/molecules26041109

[4] Maćkowiak, K., Lubawy, M., Kwiatkowska, I., Nowak-Myszka, A., Myszka, W., Gutowski, Ł., Formanowicz, D., "Spices–bioactive compounds in the diet of patients with cancer", Advances in Biomedical Research–from Cancer Prevention to Treatment, p.64.

[5] Prasad S. K., Tyagi A., "Historical spice as a future drug: therapeutic potential of piperlongumine", Current Pharmaceutical Design, vol. 1, no. 27, pp. 4151-4159, 2016. https://doi.org/10.2174/1381612822666160601103027

[6] Banwo K., Olojede A. O., Adesulu-Dahunsi A. T., Verma D. K., Thakur M., Tripathy S., Singh S., Patel A. R., Gupta A. K., Aguilar C. N., Utama G. L., "Functional importance

of bioactive compounds of foods with Potential Health Benefits: A review on recent trends", Food Bioscience, vol. 43, pp. 101320, 2021. https://doi.org/10.1016/j.fbio.2021.101320

[7] Zhang J., Hu K., Di L., Wang P., Liu Z., Zhang J., Yue P., Song W., Zhang J., Chen T., Wang Z., "Traditional herbal medicine and nanomedicine: Converging disciplines to improve therapeutic efficacy and human health", Advanced Drug Delivery Reviews, vol. 178, pp. 113964, 2021. https://doi.org/10.1016/j.addr.2021.113964

[8] Sen S., Chakraborty R., "Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future", Journal of Traditional and Complementary Medicine, vol. 7, no. 2, pp. 234-244, 2017. https://doi.org/10.1016/j.jtcme.2016.05.006

[9] Nurcholis W., Alfadzrin R., Izzati N., Arianti R., Vinnai B. Á., Sabri F., Kristóf E., Artika I. M., "Effects of methods and durations of extraction on total flavonoid and phenolic contents and antioxidant activity of java cardamom (Amomum compactum soland ex maton) fruit", Plants, vol. 11, no. 17, pp. 2221, 2022 https://doi.org/10.3390/plants11172221

[10] Anwar, F., Abbas, A., Alkharfy, K.M., "Cardamom (*Elettaria cardamomum* Maton) Oils". In Essential oils in food preservation, flavor and safety, pp. 295-301, 2016. Academic Press. https://doi.org/10.1016/B978-0-12-416641-7.00033-X

[11] Ajit A., Brijeetha P. D., Varkey P., "A mini review on the bioactive compounds in dried cardamom seed and its leaf", World J Pharm Res., vol. 9, pp. 1410-1423, 2020.

[12] Nawaz J., Rasul A., Shah M. A., Hussain G., Riaz A., Sarfraz I., Zafar S., Adnan M., Khan A. H., Selamoglu Z., "Cardamonin: A new player to fight cancer via multiple cancer signaling pathways", Life Sciences, vol. 250, pp. 117591, 2020. https://doi.org/10.1016/j.lfs.2020.117591

[13] Bano S., Majumder A., Srivastava A., Nayak K. B., "Deciphering the Potentials of Cardamom in Cancer Prevention and Therapy: From Kitchen to Clinic", Biomolecules, vol. 14, no. 9, pp. 1166, 2024. https://doi.org/10.3390/biom14091166

[14] Qiblawi S., Kausar M. A., Shahid S. M., Saeed M., Alazzeh A. Y., "Therapeutic interventions of cardamom in cancer and other human diseases", Journal of Pharmaceutical Research International, vol. 32, no. 22, pp. 74-84, 2020.

[15] Moulai-Hacene, F., Boufadi, M.Y., Keddari, S., Homrani, A., "Chemical composition and antimicrobial properties of *Elettaria cardamomum* extract", Pharmacognosy Journal, vol. 12, no. 5, pp. 1058-1063, 2020. DOI : 10.5530/pj.2020.12.149

[16] Souissi, M., Azelmat, J., Chaieb, K., Grenier, D., "Antibacterial and anti-inflammatory activities of cardamom (*Elettaria cardamomum*) extracts: Potential therapeutic benefits for periodontal infections", Anaerobe, vol. 61, p. 102089, 2020. https://doi.org/10.1016/j.anaerobe.2019.102089

[17] Velavan, S., "Phytochemical techniques-a review", World Journal of Science and Research, vol. 1, no. 2, pp. 80-91, 2015.

[18] Ahmad, A., Husain, A., Mujeeb, M., Siddiqui, N.A., Damanhouri, Z.A., Bhandari, A., "Physicochemical and phytochemical standardization with HPTLC fingerprinting of Nigella sativa L. seeds", Pakistan Journal of Pharmaceutical Sciences, vol. 27, no. 5, pp. 1175-1182, 2014.

[19] Al-Qahtani, J., Abbasi, A., Aati, H.Y., Al-Taweel, A., Al-Abdali, A., Aati, S., Yanbawi, A.N., Khan, M.A., Ghalloo, B.A., Anwar, M., "Phytochemical, antimicrobial, antidiabetic, thrombolytic, anticancer activities, and in silico studies of Ficus palmata Forssk", Arabian Journal of Chemistry, vol. 16, no. 2, pp. 104455, 2023. https://doi.org/10.1016/j.arabjc.2022.104455

[20] Amalraj, S., Krupa, J., Sriramavaratharajan, V., Mariyammal, V., Murugan, R., Ayyanar, M., "Chemical characterization, antioxidant, antibacterial and enzyme inhibitory properties of Canthium coromandelicum, a valuable source for bioactive compounds", Journal of Pharmaceutical and Biomedical Analysis, vol. 192, pp. 113620, 2021.

[21] Thangaraj, P., "Pharmacological assays of plant-based natural products", vol. 71, pp. 11-13, 2016. Berlin, Germany:Springer.

[22] Van de Loosdrecht, A.A., Beelen, R.H.J., Ossenkoppele, G., Broekhoven, M.G., Langenhuijsen, M.M.A.C., "A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia", Journal of Immunological Methods, vol. 174, no. 1-2, pp. 311-320, 1994. https://doi.org/10.1016/0022-1759(94)90034-5

[23] Fang, Y., Yang, C., Zhang, L., Wei, L., Lin, J., Zhao, J., Peng, J., "Spica Prunellae Extract Enhances Fluorouracil Sensitivity of 5-Fluorouracil-Resistant Human Colon Carcinoma HCT-8/5-FU Cells via TOP2α and miR-494", BioMed Research International, vol. 1, pp. 5953619, 2019. https://doi.org/10.1155%2F2019%2F5953619

[24] Noumi, E., Snoussi, M., Alreshidi, M.M., Rekha, P.D., Saptami, K., Caputo, L., De Martino, L., Souza, L.F., Msaada, K., Mancini, E., Flamini, G., "Chemical and biological evaluation of essential oils from cardamom species", Molecules, vol. 23, no. 11, p. 2818, 2018. https://doi.org/10.3390/molecules23112818

[25] Aghasi, Mohadeseh, Shohreh Ghazi-Zahedi, Fariba Koohdani, Fereydoun Siassi, Ensieh Nasli-Esfahani, Ali Keshavarz, Mostafa Qorbani, Hoorieh Khoshamal, Asma Salari-Moghaddam, Gity Sotoudeh. "The effects of green cardamom supplementation on blood glucose, lipids profile, oxidative stress, sirtuin-1 and irisin in type 2 diabetic patients: a study protocol for a randomized placebo-controlled clinical trial", BMC complementary and alternative medicine, vol. 18, pp. 1-6, 2018. https://doi.org/10.1186/s12906-017-2068-6

[26] Agidew, M.G., "Phytochemical analysis of some selected traditional medicinal plants in Ethiopia", Bulletin of the National Research Centre, vol. 46, no. 1, pp. 1-22, 2022. https://doi.org/10.1186/s42269-022-00770-8

[27] Ahmed, H.M., Ramadhani, A.M., Erwa, I.Y., "Phytochemical screening, chemical composition and antibacterial activity of essential oil of cardamom", World J. Pharm. Res, vol. 8, no. 9, pp. 1166-1175, 2019. https://doi.org/10.20959/wjpr20199-15504

[28] Kanthlal, S.K., Joseph, J., Paul, B.P., Vijayakumar, M., Rema Shree, A.B., Uma, D.P., "Assessment of Phytochemicals, Total Phenol, Flavonoid Content and in Vitro Antioxidant Property of Large Cardamom Extracts", Indian Drugs, vol. 58, no. 10, pp. 34-41, 2021. https://doi.org/10.53879/id.58.10.12627

[29] Fatemeh, Y., Siassi, F., Rahimi, A., Koohdani, F., Doostan, F., Qorbani, M., Sotoudeh, G., "The effect of cardamom supplementation on serum lipids, glycemic indices and blood pressure in overweight and obese pre-diabetic women: a randomized controlled trial", Journal of Diabetes & Metabolic Disorders, vol. 16, pp. 1-9, 2017. https://doi.org/10.1186/s40200-017-0320-8

[30] Yassin, M.T., Mostafa, A.A.F., Al-Askar, A.A., Alkhelaif, A.S., "In vitro antimicrobial potency of *Elettaria cardamomum* ethanolic extract against multidrug resistant of food poisoning bacterial strains", Journal of King Saud University-Science, vol. 34, no. 6, pp. 102167, 2022. https://doi.org/10.1016/j.jksus.2022.102167

[31] Poyil, M.M., Shamna, K.P., "Antibacterial Potential of Methanolic Extract of Cardamom (*E. cardamomum*) Pods Against Multidrug Resistant Klebsiella pneumoniae and Staphylococcus aureus", Applied Biological Research, vol. 24, no. 2, pp. 203-209, 2022.

[32] Molaveisi, M., Noktehsanj Avval, M., Mohammadi, M., "Chemical composition, antioxidant potential, and antimicrobial activity of *E. cardamomum* essential oil", Infection Epidemiology and Microbiology, vol. 6, no. 1, pp. 51-62, 2020.

[33] Özmatara, M.B., "Environmentally friendly extraction of antioxidants from *Elettaria cardamomum* seeds with glucose-citric acid-based natural deep eutectic solvent", Rec Agric Food Chem, vol. 1, no. 1-2, pp. 12-18, 2021.

[34] Castillo, Nora E. Torres, Giselle D. Teresa-Martínez, Maritza Alonzo-Macías, Carmen Téllez-Pérez, José Rodríguez-Rodríguez, Juan Eduardo Sosa-Hernández, Roberto Parra-Saldívar, Elda M. Melchor-Martínez, Anaberta Cardador-Martínez, "Antioxidant Activity and GC-MS Profile of Cardamom (*Elettaria cardamomum*) essential oil obtained by a combined extraction method—instant controlled pressure drop technology coupled with sonication", Molecules, vol. 28, no. 3, pp. 1093, 2023. https://doi.org/10.3390/molecules28031093

[35] Juwitaningsih, T., Jahro, I.S. and Sari, S.A., "Evaluation of North Sumatera cardamom seed (Amomum compactum) extract as antibacterial and anticancer", In Journal of Physics: Conference Series, vol. 1485, no. 1, p. 012019, 2020. https://doi.org/10.1088/1742-6596/1485/1/012019

[36] Zaki, M., Farag, H. and Abd El Wahed, S., "Anticancer Effect of Cardamom Extract on Hep-2 Cell Line", International Dental Journal, vol. 71, pp. S43-S44, 2021. https://doi.org/10.1016/j.identj.2021.08.031

[37] Pal, S., Pal, K., Mukherjee, S., Bera, D., Karmakar, P. and Sukhen, D., "Green cardamom mediated phytosynthesis of ZnONPs and validation of its antibacterial and anticancerous potential", Materials Research Express, vol. 7, no. 1, p. 015068, 2020. https://doi.org/10.1088/2053-1591/ab69c8