



***In vitro* α -glucosidase inhibitory, antioxidant and cytotoxic activities of *Cnidoscopus aconitifolius* leaf fractions from Thailand**

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Abstract

Cnidoscopus aconitifolius is a widely consumed vegetable that has potential bioactive compounds to attenuate several complex diseases, including cancer and diabetes. This study aimed to evaluate bioactivity of different polarity *C. aconitifolius* fractions, including antioxidant, α -glucosidase inhibition, and cytotoxicity on colorectal cancer cell lines. *C. aconitifolius* leaves were macerated with methanol and then partitioned with *n*-hexane and ethyl acetate, respectively, to obtain three different polarity fractions; *n*-hexane (HF), ethyl acetate (EF), and methanol (MF) fractions, which were low, medium, and high polarity fractions, respectively. The fractions were examined for antioxidant activities, antidiabetic activity, colorectal cancer cytotoxicity activities against HCT116 and SW620 cell lines, and phytochemicals. The antioxidant evaluation using the DPPH reagent indicated that the EF had the greatest scavenging ability, while the MF had the highest result against the ABTS reagent. The α -glucosidase inhibition assay using maltose substrate revealed EF had the strongest inhibitory activity, and MF had the best result with sucrose substrate. The HF inhibited HCT116 cell line development the most, whereas the EF effectively reduced SW620 cell line growth. Meanwhile, the MF had no effect against both cell lines. HF, EF and MF were analyzed for the compounds' content and found varied compounds, which should be further purified for bioactive compounds. Different polarity fractions of *C. aconitifolius* leaf showed distinct bioactivities. The characteristic of therapeutic drug discovery for cancer and diabetes diseases can be concerned with the selectable polarity of solvents.

Keywords: Colorectal cancer cytotoxicity, α -Glucosidase inhibition, Antioxidant activity, Different polarity fractions

1. Introduction

Cnidoscopus aconitifolius, a species from the Euphorbiaceae family, commonly referred to as Chaya or spinach tree, is a fast-growing and leafy perennial shrub which is believed to have originated in the Yucatán-Peninsula of Mexico. It is a popular leafy vegetable in Mexican and Central American cuisine, and demonstrates exceptional bioactive and nutritional properties within the valuable diversity of ethnomedicinal plants. This species is distributed across multiple continents, including America, Africa, and Asia [1,2]. Due to it being tasty and nutritious, *C. aconitifolius* has been introduced in Thailand and nowadays it is distributed in many provinces. In various studies, *C. aconitifolius*, additionally referred to as *Cnidoscopus chayamansa* McVaugh and *Jatropha aconitifolia* Mill., has been recognized as a globally consumed vegetable in numerous countries [3,4]. This species is proposed to contain

bioactive compounds that may alleviate the symptoms associated with chronic diseases, notably non-communicable diseases (NCDs) [5].

Diabetes and cancer are among the most prevalent and burdensome non-communicable diseases (NCDs). Oxidative stress is a key mechanism contributing to the development and progression of both conditions. In diabetes, there is an increase in superoxide levels within cells, resulting in insulin receptor inactivation [6]. A similar trend has been observed in cancer cases, where chronic inflammation caused by elevated oxidative stress plays a crucial role as a significant risk factor for cancer [7]. Furthermore, the association between diabetes mellitus and cancer has been reported as both diseases share the same risk factors, including being overweight with a body mass index (BMI) exceeding 30 [8]. In individuals with an overweight condition, chronic inflammation induced by excess fat is commonly observed, which leads to insulin resistance and stimulation of tumorigenesis. For many years, exogenous insulins have been the most frequently utilized treatment for diabetic patients, particularly those with type 2 diabetes mellitus; however, reports indicate that long-term anti-diabetic medications may have a potential impact on the development of tumorigenesis, including colorectal cancer [7,9]. Consequently, alternative medicines that utilize natural compounds provide greater safety and reduced harmful side effects, including using *C. aconitifolius* extract as a medicinal plant. The leaf is utilized to treat diabetes among traditional communities [10]. Moreover, additional studies have indicated that the extract can markedly decrease the proliferation of cancer cells and exhibit anti-inflammatory properties [11,12]. The identified compounds consist of flavonoids, phenolic acids, stilbenes, and triterpenes, which may contribute to various bioactivities [2,4].

It has been indicated that the polar solvents can improve phenolic compound extraction, which could act as effective agents for antioxidant and anticancer activities [13,14]. The extract from the non-polar solvent fraction, which includes alkaloids and terpenes, also significantly reduces in vitro cancer cell lines [15]. Another study has reported that flavone compounds extracted from medium polar to high polar solvents exhibit activities that reduce intestinal enzymes in antidiabetic assays [16]. Those findings suggested that the bioactive compounds were affected by the types of samples and solvents used [17].

Although previous studies have provided insights into the biochemical activities of *C. aconitifolius*, the consistency between bioactivities and its active compound are still less known due to *C. aconitifolius* being introduced in Thailand and the difference in the phytonutrient and phytocompound content in plant specimens depending on the plant types, extraction method, extraction solvent, planting area, crop age and post-harvest handling. The objective of this study was to analyze the antioxidant properties, α -glucosidase inhibition, cytotoxicity on colorectal cancer activities, and the phytochemicals compounds contents of different polarity fractions of *C. aconitifolius* leaf, which was obtained from Bangkok.

2. Materials and Methods

2.1 Plant material

C. aconitifolius leaves were collected from Bangkok, and identified in the Herbarium of Kasin Suvatabhandhu, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

2.2 Extraction and preparation for the fractions

The *C. aconitifolius* leaves were air-dried at room temperature and milled into powder. The 1000 g powder was macerated and extracted with methanol at ambient temperature three times, each lasting 24 hours. The combined filtrate was condensed under reduced pressure to yield the concentrated methanol crude extract. The concentrated methanol crude extract was liquid-liquid partitioned 3 times; 1:1 ratio, a time, with *n*-hexane and ethyl acetate, respectively, yielding three polarity fractions: 24.4864 g of low (*n*-hexane; HF), 23.6610 g of medium (ethyl acetate; EF), and 24.0375 g of high (methanol; MF) polarity fractions. Each fraction was collected separately and evaporated till dry. After thorough drying, the fractions were kept at -20°C for future use.

2.3 DPPH radical scavenging activity assay

The 2-fold dilutions (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, and 0.019 mg/mL) of the samples were thoroughly mixed with a 0.3 mM DPPH solution for the DPPH radical scavenging activity experiment. The mixture was then incubated in the dark at room temperature for 20 minutes. [18]. A spectrophotometer was used to evaluate the decrease in absorbance of samples compared to the DPPH-methanol mixture (control) at 517 nm. The proportion of radical inhibition was estimated using the following equation 1:

$$\text{Inhibition (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (1)$$

The absorbance of DPPH solution without the sample was used as a control, while the absorbance of the sample containing DPPH solution was used as a sample. The IC₅₀, or the amount of antioxidant necessary to reduce the initial DPPH concentration by 50%, was reported using triplicate testing. The blank was the sample solvent solution, while the negative control was the solvent with DPPH. The positive control was butylated hydroxyl toluene (BHT) with the same concentration range as the sample. All tests were performed at least three times.

2.4 ABTS radical scavenging activity assay

The radical cation of 2,2'-azino-bis-ethylbenzthiazoline-6-sulfonic acid (ABTS⁺) was generated by combining a 7 mM ABTS solution with 2.45 mM potassium persulfate at a 1:1 (v/v) ratio and allowing the mixture to stand for 4 to 16 hours until the absorbance stabilized. [19]. To achieve 0.700 ± 0.05 absorbance at 734 nm, the ABTS⁺ solution was diluted with ethanol. The photometric experiment was performed by mixing ABTS⁺ solution with the sample at various concentrations for 45 seconds before measuring it at 734 nm. All of these steps were completed in the dark. The 2-fold dilutions (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, and 0.019 mg/mL) of the samples were prepared. The antioxidant activity of the samples was calculated by comparing them to the ABTS⁺ solution at the same concentration using the DPPH equation. The sample solvent solution served as the blank, while the negative control was solvent with ABTS⁺ and the positive control was butylated hydroxyl-toluene (BHT) with the same concentration range as the sample. All tests were performed at least three times.

2.5 Rat intestinal α -glucosidase inhibitory activity assay

The inhibitory activity of rat intestinal α -glucosidase enzyme was measured using a slightly modified approach published in previous work [20]. The inhibitory activity of α -glucosidase was assessed using an α -glucosidase inhibition assay. Maltose and sucrose were used as substrates to induce maltase and sucrase, respectively. The resultant glucose can be measured using a colorimetric method. Glucose was originally oxidized in the presence of glucose oxidase to produce hydrogen peroxide (H₂O₂), which then interacts with 4-aminophenazone and phenol to produce a vivid purple solution of quinonemine. The amount of glucose was thus proportional to quinoneimine and can be evaluated by absorption at 520 nm with a microplate reader. Acarbose® was utilized as a standard positive control.

The α -glucosidase enzyme was prepared briefly as follows: 1g cold rat intestinal acetone powder (I1630, Sigma-Aldrich) was dissolved in 10 mL cold 0.1 M phosphate buffer (pH 6.8). The suspension was vortexed for 20 seconds and ultrasonicated for 10 seconds, with cooling cycles on ice 5-10 times to fully activate the enzyme. Then, the enzyme suspension was subsequently centrifuged at 12000xg at 4°C for 30 minutes; after that, the supernatant was stored at -80°C until use. All preparation processes were performed on ice.

The α -glucosidase inhibition experiment was investigated using sucrose substrates to induce sucrose and maltose substrates to induce maltase. The 2-fold dilutions (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, and 0.019 mg/mL) of the samples were prepared. Acarbose was a positive control with the same concentration range as the sample. All tests were performed at least three times.

In a 96-well plate, mix 10 μ L of sample with 30 μ L of 0.1 M phosphate buffer (pH 6.8), 20 μ L of substrate solution (10 mM maltose or 100 mM sucrose), and 20 μ L of enzyme. The reaction mixture was then incubated at 37 °C (10 minutes for maltose and 40 minutes for sucrose). The resultant glucose can be measured using a colorimetric method. The glucose oxidase method was used to determine the reaction mixture, with 80 μ L of a glu-kit (Human, Germany). Absorbance at 520 nm was used to quantify enzymatic activity. The percentage inhibition value was calculated using equation 2:

$$\text{Inhibition (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (2)$$

Where A control was the absorbance without the sample and A sample was the absorbance with the sample. The IC₅₀ value was be calculated using a plot of percentage inhibition and final response concentration. The experiment was carried out in triplicate.

2.6 Cell lines preparation and culture

The colorectal cancer cell lines, HCT116 and SW620, were used. The cells were initially grown in Gibco Dulbecco's Modified Eagle Medium (DMEM) at 37°C in a humid environment containing 5% CO₂. The cells went through two passages before being used in the experiment [21]. SW620 was bought from ATCC, HCT116 was generously donated by Chulalongkorn University's Center of Excellence in Molecular Genetics of Cancer and Human Disease, and authentication was accomplished using STR profiling.

2.7 Cytotoxicity on colorectal cancer cell lines (MTT Assay)

The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was performed to determine the percentage of cell viability. Following prior work, 5x10³ cells/well were grown in a 96-well plate for 24 hours before adding fresh media containing various concentrations of the samples. The 2-fold dilutions (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, and 0.019 mg/mL) of the samples were prepared. The cells were then incubated for 48 hours before being measured at 570 nm using an ELISA reader. The negative control was an untreated cell with DMEM, and the positive control was a cisplatin-treated cell. Each treatment was performed in triplicate. The absorbance data was presented as a percentage viability compared to untreated cells. The acquired data was utilized to compute the fraction of viable dead cells using equation 3, as published in prior work [22].

$$\text{Cell viability (\%)} = \frac{(\text{Abs of sample})}{\text{Abs control}} \times 100 \quad (3)$$

The absorbance of treated cells was denoted as Abs of the sample, while the absorbance of untreated cells was denoted as Abs of the control. The compound concentration necessary to inhibit cell proliferation by 50% was designated as the half-maximal inhibitory concentration (IC₅₀). The IC₅₀ was determined by generating curves that plotted cell survival against sample concentration. The negative control was an untreated cell with DMEM; the blank was DMEM, and the positive control was a cisplatin-treated cell with DMEM. [22].

2.8 GC-MS (Gas Chromatography-Mass Spectrometry) analysis

2.8.1 Preparation of Samples

For GC-MS analysis, the *C. aconitifolius* fractions solution of 200 µg/mL in 1 mL was used. The samples were prepared as follows: the *n*-hexane fraction (HF) was dissolved in *n*-hexane, the ethyl acetate fraction (EF) was dissolved in ethanol, and the methanol fraction (MF) was dissolved in methanol.

2.8.2 GC-MS (Gas Chromatography-Mass Spectrometry) analysis

The phytochemicals investigation of *C. aconitifolius* fractions was performed on GC-MS equipment (SCION 8700 Single Quad GC-MS). Experimental conditions of the GC-MS system were as follows; the instrument was equipped with a Scion-5MS 30 m x 0.25 mm ID, 0.25 µm film thickness. The flow rate of the mobile phase (carrier gas: helium) was set at 1.0 mL/min. The temperatures were employed as 80 °C column oven temperature and 250 °C injection temperature. The GC program ion source and interface temperature were 200.00 °C and 250.00 °C, respectively, with a solvent cut time of 2.50 min and an injection volume of 1 µL of the samples (split ratio 10:1). The MS program started at 3.00 min, ended at 45.00 min with an event time of 0.50 sec, and had a scan range of 30-500 m/z. The total running time of GC-MS was 45 min. The results were compared using the Wiley Spectral Library Search program NIST MS Search 2.0. The relative percentage of the fractions was expressed as a percentage with peak area normalization.

2.9 Data and statistical analysis

Each treatment was administered in a minimum of three replicates. SPSS (IBM Corp.) was employed to conduct a one-way analysis of variance, which was subsequently followed by Duncan's test for significance, in order to examine the differences between the groups. Statistical significance was determined by P-values that were less than 0.05. The data were presented as mean ± standard deviation (SD).

3. Results

3.1 Antioxidant evaluation using DPPH and ABTS radical scavenging assay

The antioxidant evaluation of *C. aconitifolius* leaf fractions were assessed using DPPH and ABTS radical scavenging assays, as the results summarize in Table 1. In terms of the half maximal inhibitory concentration (IC_{50}) results, DPPH results demonstrated that EF showed the highest scavenging ability with the lowest IC_{50} (1.369 ± 0.035) $\times 10^3$ μ g/mL), followed by MF (7.932 ± 0.162) $\times 10^3$ μ g/mL) and HF (22.167 ± 0.622) $\times 10^3$ μ g/mL). However, ABTS results demonstrated that MF showed the highest scavenging ability with the lowest IC_{50} (1.262 ± 0.0284) $\times 10^3$ μ g/mL), followed by EF (1.300 ± 0.227) $\times 10^3$ μ g/mL) and HF (38.082 ± 0.315) $\times 10^3$ μ g/mL).

Table 1 Antioxidant evaluation of *Cnidoscolus aconitifolius* leaf fractions

Fractions	IC_{50} of DPPH (μ g/mL)	IC_{50} of ABTS (μ g/mL)
<i>n</i> -hexane (HF)	$(22.167 \pm 0.622) \times 10^3$ ^c	$(38.082 \pm 0.315) \times 10^3$ ^c
Ethyl acetate (EF)	$(1.369 \pm 0.035) \times 10^3$ ^a	$(1.300 \pm 0.227) \times 10^3$ ^b
Methanol (MF)	$(7.932 \pm 0.162) \times 10^3$ ^b	$(1.262 \pm 0.284) \times 10^3$ ^a
BHT as positive control	$(118.331 \pm 17.188) \times 10^3$ 0.537 $\pm$ 0.078 mM	$(198.541 \pm 1.542) \times 10^3$ 0.901 $\pm$ 0.007 mM

One-way ANOVA with Duncan test, p -value <0.05 ; sample size(n)=3. The data are presented as mean $\pm$ standard deviation (SD). The subscribed letter indicated the significant value between fractions in each group DPPH or ABTS. BHT: butylated hydroxyl-toluene as positive control.

3.2 Antidiabetic evaluation using rat intestinal α -glucosidase inhibition assay

Antidiabetic evaluation of the fractions were assessed using rat intestinal α -glucosidase inhibition assay with sucrose and maltose as substrates. The results are summarized in Table 2. The experiment that employed maltose as a substrate to induce maltase demonstrated that the EF exhibited the highest inhibitory activity with the lowest IC_{50} ($(6.181 \pm 0.041) \times 10^3$ μ g/mL), followed by the MF ($(11.886 \pm 0.077) \times 10^3$ μ g/mL) and the HF ($(25.912 \pm 0.043) \times 10^3$ μ g/mL). When sucrose was utilized as a substrate to induce sucrase, the MF had the highest inhibitory effectiveness with the lowest IC_{50} ($(0.286 \pm 0.066) \times 10^3$ μ g/mL), followed by the EF ($(21.101 \pm 0.021) \times 10^3$ μ g/mL) and the HF ($(47.564 \pm 0.085) \times 10^3$ μ g/mL).

Table 2 Antidiabetic evaluation using rat intestinal α -glucosidase inhibitory activation assay of *Cnidoscolus aconitifolius* leaf fractions

Fractions	IC_{50} of maltose as substrate (μ g/mL)	IC_{50} of sucrose as substrate (μ g/mL)
<i>n</i> -hexane (HF)	$(25.912 \pm 0.043) \times 10^3$ ^c	$(47.564 \pm 0.085) \times 10^3$ ^c
Ethyl acetate (EF)	$(6.181 \pm 0.041) \times 10^3$ ^a	$(21.101 \pm 0.021) \times 10^3$ ^b
Methanol (MF)	$(11.886 \pm 0.077) \times 10^3$ ^b	$(0.286 \pm 0.066) \times 10^3$ ^a
Acarbose as positive control	$(41.319 \pm 5.810) \times 10^3$ 0.064 $\pm$ 0.009 mM	$(157.527 \pm 40.027) \times 10^3$ 0.244 $\pm$ 0.062 mM

One-way ANOVA with Duncan test, p -value <0.05 ; sample size(n)=3. The data are presented as mean $\pm$ standard deviation (SD). The subscribed letter indicated the significant value between fractions in each group maltase substrate or sucrose substrate. Acarbose as positive control.

3.3 Cytotoxicity on colorectal cancer cell lines using MTT assay

The Cytotoxicity of the fractions against HCT116 and SW620 cell lines was evaluated by IC_{50} determination (Table 3). The HF inhibited cell viability for HCT116 with the lowest IC_{50} (12.337 ± 0.780 μ g/mL), followed by the EF (12.961 ± 0.391 μ g/mL); however, the MF did not inhibit HCT116. The EF inhibited SW620 cell viability the most, with the lowest IC_{50} (21.050 ± 0.097 μ g/mL), followed by the HF (48.562 ± 0.222 μ g/mL); however, the MF showed the result similar to HCT116 that had no activity against SW620.

Table 3 Cytotoxicity on colorectal cancer cell lines of *Cnidoscolus aconitifolius* leaf fractions

Fractions	IC_{50} against HCT116 (μ g/mL)	IC_{50} against SW620 (μ g/mL)
<i>n</i> -hexane (HF)	12.337 ± 0.780 ^a	48.562 ± 0.222 ^b
Ethyl acetate (EF)	12.961 ± 0.391 ^b	21.050 ± 0.097 ^a
Methanol (MF)	ND	ND
Cisplatin as positive control	$(0.903 \pm 0.301) \times 10^3$ 0.003 $\pm$ 0.001 mM	$(2.408 \pm 0.602) \times 10^3$ 0.008 $\pm$ 0.002 mM

One-way ANOVA with Duncan test, p -value <0.05 ; sample size(n)=3. The data are presented as mean $\pm$ standard deviation (SD). The subscribed letter indicated the significant value between fractions in each group HCT116 or SW620. ND: non detectable. Cisplatin as positive control.

3.4 Phytochemicals investigation using GC-MS

The GC-MS chromatogram of the of *C. aconitifolius* fractions was analyzed. The *n*-hexane fraction (HF) of *C. aconitifolius* showed many compounds and the components corresponding to the peaks were determined as follows; Toluene (1)(7.6%), 2,4- Dimethyl- 1- heptene (3)(4.5%), 2- Methyl-2- pentanol (8)(7.3%), 2- Methyl-3- hexanol (10)(3.7%), 1- Methylcyclopentanol (14)(3.7%), and other compounds showed low of percentage of peak area (Fig 1). Nine compounds were analysed in the ethyl acetate fraction (EF). These constituents and their calculated percentage peak area compositions include Triethoxymethylsilane (3)(2.9%), *n*-Decane (7)(3.7%), Undecane (9)(8.5%), Dodecene (11)(4.2%), 1,3-Ditertiarybutylbenzene (14)(5.6%), 2,4- Di-tert-butylphenol (17)(21.3%), D-Friedoolean-14-en-3-one (28)(6.2%), 3-O-(2-acetoxy)ethyl-cholesterol (29)(3.1%), Olean-12-en-3-ol, acetate (30)(5.3%), and other compounds showed low of percentage of peak area (Fig 2). Seven compounds were analyzed in the methanol fraction (MF) as follows; 1-Dodecene (1)(6.5%), 1,3-Di-tert-butylbenzene (3)(4.1%), 1-Tridecene (5)(8.5%), 2,4-Di-tert-butylphenol (6)(46.4%), 1-Tetradecene (7)(4.3%), Hexadecanoic acid methyl ester (13)(12.0%), Heptadecanoic acid 16-methyl (16)(5.7%), and other compounds showed low of percentage of peak area (Fig 3).

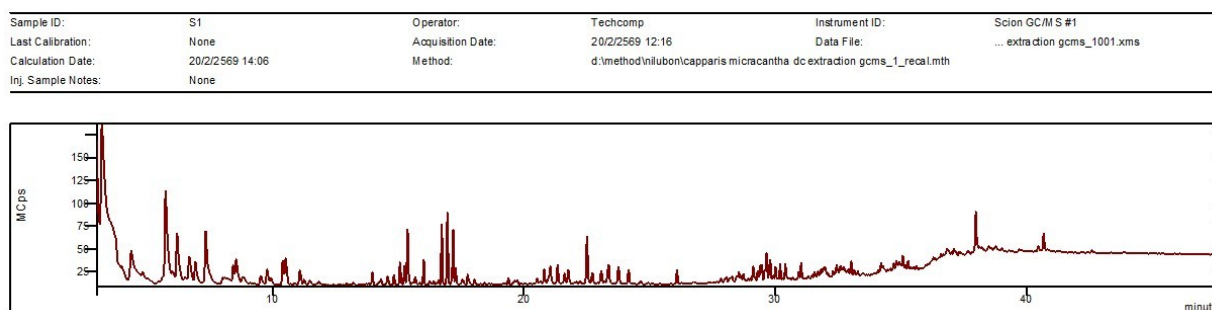


Figure 1 GC-MS Chromatogram of *n*-hexane fraction (HF) of *Cnidoscopus aconitifolius* leaf fractions

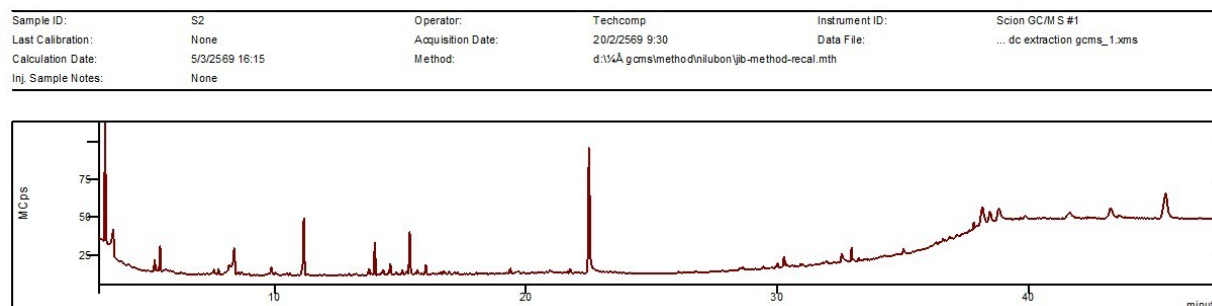


Figure 2 GC-MS Chromatogram of ethyl acetate fraction (EF) of *Cnidoscopus aconitifolius* leaf fractions

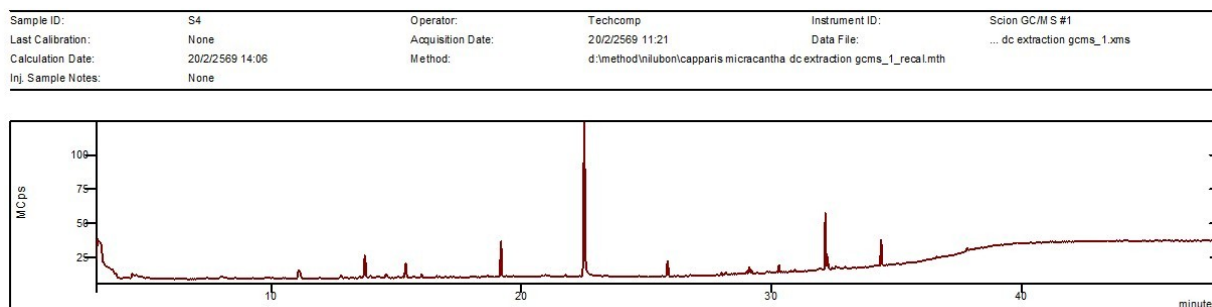


Figure 3 GC-MS Chromatogram of methanol fraction (MF) of *Cnidoscopus aconitifolius* leaf fractions

4. Discussion

One of the major causes of death affecting millions of people is cancer, which is caused by the uncontrolled manner of abnormal cells. The International Agency for Research on Cancer (IARC) estimated that 12.7 million new cancer cases and 7.6 million cancer deaths occurred worldwide in 2008 [23]. Presently, cancer therapy depends on surgery, radiotherapy, and chemotherapy; nevertheless, it is regrettable that there are a significant number of patients who die from these effects [24]. Colorectal cancer was focused on because it is the third most prevalent cancer worldwide, accounting for more than 1.2 million patients each year; it is also the third most common cancer in women and the fourth in men. One treatment guideline to reduce the adverse effects of therapy is identifying compounds that can be used as conjunction with cancer therapy as adjuvants. Presently, there are several studies to investigate more effective, safer, and inexpensive anti-cancer medications from alternative sources such as bioactive chemicals from plants [25,26]

Diabetes mellitus affects individuals globally and is estimated to reach at least 366 million by 2030. Type 2 diabetes mellitus (T2DM) is a chronic metabolic condition characterized by hyperglycemia or elevated blood glucose levels. T2DM is caused by the lack of insulin secretion and the resistance to the insulin action in various tissues, resulting in impaired glucose uptake [27–29]. Delaying glucose absorption via the α -glucosidase enzyme in the digestive organ is a therapeutic method to cure diabetes and prevent complications. α -Glucosidase inhibitors as treatment drugs, including acarbose, voglibose, and miglitol, are competitive inhibitors of intestinal α -glucosidase and reduce the postprandial digestion and absorption of disaccharides; however, some drugs have side effects [30]. All over the world for centuries, plants have been used in diabetes mellitus treatment [31]

Reactive oxygen species (ROS) and free radicals induce denaturing processes in numerous cellular components, causing oxidative stress and resulting in NCDs such as cancer and diabetes [32]. There is a complicated reciprocation between the ROS and antioxidants in cancer and diabetes, which leads to the concern about the oxidative stressors that impact the imbalance of the ROS and its oxidants. [33]. Furthermore, an increase in free radical generation and a decrease in antioxidant defense may contribute to the onset and development of cancer and diabetes-related problems. According to studies, ROS has the potential to target numerous therapeutic drugs and enhance pro-tumorigenic signaling in tumor microenvironments by encouraging cancer cell development. In addition, in diabetes patients, the chronic exposure to hyperglycemia is a major causative factor to induce oxidative stress through overproduction of ROS, which may be a contributory factor to several complications from cell injury and tissue damage [34].

The creation of natural and safe solutions with minimal adverse effects is becoming increasingly vital. Plant phytochemical components have sparked increased interest in the prevention and treatment of a variety of ailments, including cancer and diabetes, due to their abundance of natural bioactive compounds, secondary metabolites, and antioxidants. Healing with medicinal plants is as ancient as humanity itself. In Mexico and Central America, especially in developing regions, among the ethnomedicinally important plants which are well known in the traditional system of medicine are the plants in the *Cnidioscolus* genus [35]. *Cnidioscolus aconitifolius* has been reported to exhibit numerous bioactivities; however, the consistency between bioactivities and active chemicals is still unclear. [36]. Plant kinds, extraction technique, extraction solvent, planting area, crop age, and post-harvest management all have an impact on phytocompound synthesis, but the extraction solvent and method are also crucial.[4].

Due to *Cnidioscolus aconitifolius* being a herbal plant which was introduced to Thailand as a leafy vegetable, it was collected from Bangkok to examine the compounds' contents, antioxidant properties, α -glucosidase inhibition, and cytotoxicity on colorectal cancer activities of different polarity fractions. This research method depended on the principle that the crude extract was liquid-liquid partitioned with different polarity solvents to separate and obtain the different polarity fractions, which contained the different polarity compounds.

Therefore, the concentrated methanol crude extract of macerated dry leaves of *C. aconitifolius* was liquid-liquid partitioned with *n*-hexane and ethyl acetate, respectively, yielding three polar fractions: 24.4864 g of low (*n*-hexane; HF), 23.6610 g of medium (ethyl acetate ; EF), and 24.0375 g of high (methanol; MF) polarity fractions. These three fractions were dried and kept at -20°C for determination with 4 methods: 1) GC-MS to identify the compounds content; 2) DPPH and ABTS radical scavenging activity for antioxidant activity; 3) rat intestinal α -glucosidase inhibition activities against maltase and sucrose; and 4) MTT assay against 2 colorectal cancer cell lines, HCT116 and SW620.

The antioxidant activities of leaf fractions are represented in Talbe 1. EF stated the greatest DPPH radical scavenging activity, followed by MF and HF. MF exhibited the greatest ABTS radical scavenging activity, followed by EF and HF. The results suggested that HF had the lowest antioxidant activities, about 20-30-fold compared to the others. Thus, this suggested that EF a

α -Glucosidase inhibitory activities of the fractions, as shown in Table 1, showed the following: EF showed the best inhibition against maltase, followed by MF and HF. MF showed the best inhibition against sucrose, followed by

EF and HF. The results suggested that HF had the lowest α -glucosidase inhibitory activity against both maltase and sucrase. HF had lower α -glucosidase inhibitory activity against maltase, about 4-fold compared to EF and about 2-fold compared to MF. In addition, HF had the lower α -glucosidase inhibitory activity against sucrase about 2-fold compared to EF and especially about 40-fold compared to MF. Moreover, the α -glucosidase inhibitory activity against maltase results showed the same trend as DPPH radical scavenging activity, in which EF showed the best activity, followed by MF and HF. Furthermore, the α -glucosidase inhibitory activity against sucrase results were the same linearity as ABTS radical scavenging activity, in which MF showed the best activity, followed by EF and HF.

The HCT116 and the SW620 colorectal cell lines were used for the cytotoxicity of the fractions. The HCT116 cell line is a human colorectal carcinoma cell line derived from a colon tumor of a male patient, whose stable genetic profile and capacity to develop tumors and metastases in immunocompromised mice make it a popular model in cancer research, especially for the study of colon cancer [37]. The SW620 cell line is a human colorectal adenocarcinoma cell line which was obtained from a Dukes' C colorectal cancer patient's lymph node metastases. The SW620 cell line is a widely used model in cancer research, especially for investigating drug discovery, metastasis, treatment resistance, and cell signaling pathways that contribute to the advancement of cancer [38].

Cytotoxic activities against colorectal cancer cell lines of the fractions, as shown in Table 3, indicate the following: HF and EF exerted a potent cytotoxic effect against HCT116 and SW620 cancer cell lines. This is noteworthy, as HF exerted a potent cytotoxic effect against SW620 better than EF, at about 2-fold. Importantly, MF had no-potential cytotoxic effect against both HCT116 and SW620 cancer cell lines. It is interesting that the cytotoxic activities results were different from the antioxidant and α -glucosidase inhibitory activities.

The GC-MS chromatogram results of the of *C. aconitifolius* fractions were analyzed. The identified compounds of HF mainly belonged to aromatic hydrocarbon, alkene and alcohols, with many compounds such as Toluene (1), 2,4-Dimethyl-1-heptene (3), 2-Methyl-2-pentanol (8), 2-Methyl-3-hexanol (10), 1-Methylcyclopentanol (14), and other compounds showed low of percentage of peak area. The identified compounds of EF mainly belonged to hydrocarbons, aromatic compounds, phenolic compounds, terpenoids, triterpenoids, steroids and sterols, including Triethoxymethylsilane (3), n-Decane (7), Undecane (9), Dodecene (11), 1,3-Ditertiarybutylbenzene (14), 2,4-Di-tert-butylphenol (17), D-Friedoolean-14-en-3-one (28), 3-O-(2-acetoxy)ethyl-cholesterol (29), Olean-12-en-3-ol, acetate (30), and other compounds showed low of percentage of peak area. The identified compounds of MF mainly belonged to alkenes, aromatic hydrocarbons, phenolic compounds, fatty acids and fatty acid derivatives, such as 1-Dodecene (1), 1,3-Di-tert-butylbenzene (3), 1-Tridecene (5), 1-Tetradecene (7), Hexadecanoic acid methyl ester (13), Heptadecanoic acid 16-methyl (16), with 2,4-di-tert-butylphenol (6) (46.4%) being the major component. Some of these compounds may response the bioactivity. Therefore, further study of *C. aconitifolius* fractions should further separate to find out the active compound of each bioactivity depend on its component and bioactivity assay.

Previous studies reported that the genus *Cnidioscolus* acetone extract, which is a semi-polar solvent, contained phenolic compounds such as flavonoids, coumarin, phenols, and tannins; moreover, those compounds are similarly present in the ethanol solvent, which is a polar solvent [39, 40]. Another study stated that antioxidant properties are important to attenuate NCDs, including chronic diseases such as diabetes and cancer [41]. Previous investigations utilizing high-performance liquid chromatography (HPLC) found that the ethyl acetate extract of *C. aconitifolius* contained an abundance of flavonoids, particularly kaempferol, quercetin, and rutin [42, 43]. Flavones have been shown to inhibit the enzyme α -glucosidase. According to studies, quercetin inhibits the enzyme's active site competitively by forming two hydrogen bonds there. The -OH groups in the B-ring can block the α -glucosidase enzyme. Inhibiting intestinal enzymes reduces glucose release from starch (polysaccharides), which results in delayed glucose absorption via the intestinal lumen [44]. In the past, studies revealed that the hexane extract of *C. aconitifolius* had the presence of terpenes and sterols [45]. Furthermore, terpenoid groups, such as triterpenoids and carotenoids, have been shown to overcome multidrug resistance in cancer cells by inhibiting p-glycoprotein overexpression, indicating that they are suitable candidates for co-administration with chemotherapy drugs [46]. In addition, phytosterols are reported to have an anticancer effect, including for colorectal cancer [47].

As a case in point, the study natural products derived from higher plant species; *Boesenbergia rotundo* Linn. found that the ethanol fraction, high-polar fraction, indicated significantly higher potential antifungal activity than the dichloromethane fraction, which is a semi-polar fraction. [48]. In a similar case, the research compared the effectiveness of *Zingiberaceae* species and demonstrated that dichloromethane fraction, a semi-polar fraction from galangal, exhibited significantly higher inhibition against *Rhizoctonia solani* Kuhn; a pathogenic fungus, than the methanol fraction which is high-polar fraction [49]. Hence, the different polarity of solvent extraction is supposed to have different biocompounds gained [50]. The methodology, such as extraction and liquid-liquid partition with different polarity solvents, was utilized with the *Coccinia grandis* stem introduced to further fractionate based on bioactivity assay till the active pure compounds were obtained and further study its enzyme kinetic manner [51]. Thus, similar to *C. grandis* stem methodology, this study on *C. aconitifolius*, an introduced plant in Thailand, demonstrated

that the bioactivity of leaf fractions varies with solvent polarity. These findings suggest that optimizing extraction and partition solvents can selectively enrich bioactive compounds, allowing for the development of fractions with targeted antioxidant, antidiabetic, or anticancer activities; however, further study should be done to find out the pure active compounds as well as pharmacodynamics studies to increase its scientific understanding and medicinal implications.

5. Conclusion

The objectives of this research were to analyze the antioxidant properties, α -glucosidase inhibition, cytotoxicity on colorectal cancer activity, and the phytochemical compounds content of different polarity fractions of *C. aconitifolius* leaf. The *n*-hexane (HF) or low polarity fraction, which mainly belongs to aromatic hydrocarbons, alkenes and alcohols, showed the best cytotoxicity on colorectal cancer activity against HCT116 cell lines. The ethyl acetate (EF) or medium polarity fraction, which mainly belonged to hydrocarbons, aromatic compounds, phenolic compounds, terpenoids, triterpenoids, steroids, and sterols, indicated the best antioxidant activity with the DPPH reagent and had the greatest α -glucosidase inhibitory activity against maltase; moreover, it showed the highest cytotoxicity on colorectal cancer activity against the SW620 cell line. The methanol (MF) or high polarity fraction which mainly belonged to alkenes, aromatic hydrocarbons, phenolic compounds, fatty acids and fatty acid derivatives had the strongest antioxidant activity with ABTS reagent and had the strongest α -glucosidase inhibitory activity against sucrose, but had no cytotoxicity on colorectal cancer effect against both cell lines. Some of these compounds may respond to the bioactivities.

The results implied that the *C. aconitifolius* leaf extracted fraction had potent agents as antioxidants, α -glucosidase inhibitors, and cytotoxic agents on colorectal cancer. These findings suggest that optimizing extraction and partition solvents could selectively enrich bioactive compounds, allowing for the development of fractions with targeted activities; therefore, further research should identify the active compounds as well as pharmacodynamics studies to increase its scientific understanding and medicinal implications.

6. Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work the author(s) used Quillbot in order to make good sentences with minimum grammatical error. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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8. Authors contributions

PU and PY contributed to the study conceptualization. PU, NS, and AJS carried out the experiment. PU, NS, AJS, and PY analyzed and validated the data. PU, NS, and AJS wrote the draft manuscript. PY supervised the study and edited the manuscript. PU, NS, and PY acquired the funding and resources. All authors have read and approved the final manuscript.

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