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In vitro α-Glucosidase Inhibitory, Antioxidant and Anti-Colorectal Cancer Activities of Gourd (*Coccinia grandis*) Stems Fractions and its Compounds

Pataraporn Uaraksakul[1,2], Nilubon Sornkaew[3], Preecha Phuwapraisirisan[4], Alvi J. Syukriya[1], Pattamawadee Yanatatsaneejit[5]*

[1]*Program in Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*Program in Medical Biology, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok 10600, Thailand*
[3]*Herbal and Cannabis Science program, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok 10600, Thailand*
[4]*Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[5]*Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

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ABSTRACT

Plant-derived agents from a medicinal plant *Coccinia grandis*, are known to have a considerably effect on several bioactivities. This present study evaluated the identified compounds on stem fractions of *C. grandis* through their antioxidant, anti-diabetes mellitus, and anti-colorectal cancer properties. Dried of *C. grandis* stems from Thailand were macerated with methanol and then fractionated with n-hexane and ethyl acetate. Each fraction was then assayed for antioxidant activities, anti-diabetes agent using rat intestinal α-glucosidase, and anti-colorectal cancer properties on HCT116 and SW620 cell lines. Ethyl acetate fractions were then subjected to further fractionation and compound identification. There were 7 identified compounds including caffeic acid, p-hydroxycoumaric acid, ferulic acid, 4-hydroxybenzaldehyde, methyl caffeate, syringaldehyde and vanillic acid. Methyl caffeate (IC_{50}= 0.145mM) and ferulic acid (IC_{50}= 0.057mM) have significant DPPH and ABTS radical scavenging activity, respectively. Caffeic acid had the strongest α-glucosidase inhibitory activity through both maltase (IC_{50}= 0.732mM) and sucrase (IC_{50}= 0.111mM), however it was not all that different from methyl caffeate. Methyl caffeate (IC_{50}= 0.001mM) and caffeic acid (IC_{50}= 0.085mM) exhibited anti-colorectal cancer effects against HCT116 and SW620, respectively. The kinetic investigation of methyl caffeate indicated that it retarded maltase function in a competitive manner and sucrase function in an uncompetitive manner. Regarding the results, it demonstrated that certain active compounds of *C. grandis* stems have their promising action on those bioactivities assay and have potent charges as drug candidates against oxidative stress, diabetes mellitus, and colorectal cancer diseases.

Keywords: Active compounds, Antioxidant, Anti-diabetes mellitus, Anti-colorectal cancer, *Coccinia grandis* stems

Introduction

Diabetes mellitus and cancer are worldwide public health problems. Both diseases have the same probable risk factors, which were classified into two groups: non-modifiable and modifiable risk factors. Age, gender, and ethnicity were non-modifiable risk factors, whereas modifiable risk factors included a non-healthy diet habit that can lead to obesity, low physical activity, and a non-healthy social lifestyle such as excessive alcohol consumption and smoking, which can increase reactive oxygen species (ROS) formation.[1,2] Currently, pharmacologic agents such as acarbose and cisplatin are available to treat diabetes and cancer, respectively. However, adverse effects may persist after long-term treatment; thus, there has been an attempt to research natural products that have these effects, providing safer agents that are less harmful. Medicinal plants have an essential role in the lives of rural people, especially those living in remote areas of developing nations with limited access to good health care.[3]

*Corresponding author. E mail: Pattamawadee.Y@chula.ac.th
Tel: +662-2185485

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Historically, herbal medicines have been used to treat a variety of illnesses ranging from mild to severe.[4] *Coccinia grandis* is a plant in the Cucurbitaceae family that has been traditionally utilized as a medicinal herb by folk healers in Bangladesh, China, India, the Philippines, Indonesia, and Thailand.[5,6] Almost every part of this plant has been shown to contain therapeutic compounds for treating oxidative stress, inflammation, obesity, and cancer, except the stems, with still less studies.[7,8] A previous research demonstrated that methanol fruit extracts of *C. grandis* have free radical scavenging action due to their flavonoid content.[9] The presence of polyphenolic chemicals in *C. grandis* leaves and fruits has been attributed to its potent antidiabetic action.[10] Furthermore, ethanolic extract of *C. grandis* leaves greatly decreased the viability of cancer cells in mice, and had an effective therapeutic agent for diabetes and diabetes-related illnesses such as oxidative stress and cancer.[11,12] However, there is a lack of information regarding how to use the *C. grandis* stems and its different polar fractions. This study aimed to observe the different polar fractions and the active compounds of *C. grandis* stems in several bioactivities such as antioxidant against 2,2-azino-bis(3-ethilbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, *in vitro* anti-diabetes mellitus using rat intestinal α-glycosidase enzyme with maltose and sucrose, and anti-colorectal cancer using HCT116 and SW620 cell lines.

Materials and Methods

Plant material

The *C. grandis* stems was collected from natural habitat in Nakhon Ratchasima province, Thailand. The herb was identified by Herbarium

of Kasin Suvatabhandhu, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.

Extraction and preparation of Coccinia grandis fractions

The *C. grandis* stems were air-dried at room temperature and milled into powder. The 1.7 kg powder was macerated with 95% methanol three times at room temperature for 24 hours a time. The filtrate was concentrated under reduced pressure using rotary evaporator. The extraction was liquid-liquid partitioned with n-hexane and ethyl acetate (EtOAc), respectively, resulting in three different polar fractions from low- semi- and high- polar fractions: *n*-hexane (10.25g), ethyl acetate (14.10g), and methanol (0.80g) fractions. The organic layer portions were collected separately, and evaporated. After thorough drying, the fractions were kept at -20°C for further study. Based on polarity and rat intestinal α-glucosidase inhibitory assay guide, the fraction was further separated using a mix of column chromatographic techniques including silica gel column chromatography, sephadex LH-20 column chromatography, and thin layer chromatography (TLC). The active chemicals were characterized using spectroscopic techniques.

The α-glucosidase inhibition assay (Bioassay-guided isolation)

The rat intestinal α-glucosidase enzyme inhibition activity was done using the method published by Osiako et al., (2023) [13], with minor modifications. The activity was investigated using maltose and sucrose as substrates to induced maltase and sucrase, respectively. The resultant glucose was measured using a colorimetric method. Briefly, 10µL of sample was applied to a 96-well plate along with 30µL of 0.1M phosphate buffer (pH 6.9) and 20µL of substrate solution (maltose 10mM, sucrose 100mM). The reaction mixture is then incubated at 37°C for glucose oxidase determination with 80µL a glucose-kit (Human, Germany). Acarbose was utilized as a positive control in this test. The absorbance was measured at 520nm to determine enzymatic activity. The % inhibition and IC_{50} values are computed as follows:

$$\text{Inhibition (\%)} = \frac{(A\,0 - A\,1)}{A\,0} \text{x}100 \qquad (1)$$

Where: A0 is the absorbance without the sample, and A1 is the absorbance with the sample. The IC_{50} value is calculated by plotting the percentage inhibition against the final concentration of the reaction. Acarbose provides positive control. The experiment is carried out in triplicate.

Separation, purification and characterization of isolated compounds

The ethyl acetate extract (EtOAc, EA) 14.10 g was fractionated by column chromatography using a gradient solvent system of *n*-hexane–EtOAc (80:20), (70:30), (50:50) and EtOAc with increasing amounts of polar solvent. The eluates were examined by TLC and four combined fractions (EA1-EA4) were obtained. Fraction EA1 was further purified using column chromatography using *n*-hexane-EtOAc (80:20) to give compound 1 (45.5mg). Fraction EA2 was purified similarly, affording compound 2 (32.8mg). Fraction EA3 was subjected to sephadex LH-20 column chromatography using 100% methanol to afford three combined fractions (EA3.1-EA3.3).

Fraction EA3.2 was subjected to column chromatography using sephadex LH-20 column chromatography using 100% methanol to afford 5 combined fractions (EA3.2.1-EA3.2.5). Fraction EA3.2.3 was isolated by column chromatography on silica gel using *n*-hexane-EtOAc (60:40) and sephadex LH-20 to afford compound 3 (71.5mg), compound 4 (21.4mg), and compound 5 (110.7mg), respectively. Fraction EA3.2.4 was subjected to column chromatography on silica gel using *n*-hexane-EtOAc (100:10, 100:20, 100:50) with increasing amounts of the more polar solvent and using sephadex LH-20 to afford compound 6 (16.0mg) and compound 7 (20.7mg), respectively. Identification of the chemical structures by $^{13}C/^{1}H$- Nuclear Magnetic Resonance (NMR) spectroscopy. In this study, ^{1}H and ^{13}C NMR were recorded on a Bruker Avance NMR spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz.

Evaluation of the mechanism of the α-glucosidase inhibition

The Lineweaver-Burk equation and secondary plots were used to analyze the inhibitory mechanism of *α*-glucosidase inhibitors. To assess the kinetic mode of inhibition against *α*-glucosidase, isolated compound was tested in a series of solutions with varying inhibitor concentrations (for against maltase, the series concentration were 0, 2.5, 5, and 10 mM; while, for against sucrase were 0, 2.5, 3, and 5 mM). The antidiabetic activity was assessed by an *α*-glucosidase inhibition experiment with sucrose and maltose as substrates. The resultant glucose was measured using a colorimetric method. Glucose was first oxidized by glucose oxidase to produce hydrogen peroxide (H_2O_2), which then interacts with 4-aminophenazone and phenol to produce a vivid purple solution of quinoneimine. The amount of glucose is thus proportional to quinoneimine and can be evaluated by absorption at 520nm with a microplate reader. Acarbose was utilized as the standard control. The kinetic investigation will use Lineweaver Burk plot parameters to measure the concentration of substrates (maltose and sucrose) and rat intestinal α-glucosidase in the absence and presence of tested substances. Data from kinetic studies provide insights into the inhibition mechanism [14]. The Lineweaver Burk plot equation in double reciprocal form is represented as:

$$\frac{1}{v_o} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}} \qquad (2)$$

Secondary plots can be constructed from:

$$Slope = \frac{K_m}{V_{max}} + \frac{K_m[I]}{V_{max}K_i} \qquad (3)$$

$$Y - intercept = \frac{1}{V_{max}} + \frac{[I]}{\alpha K_i' V_{max}} \qquad (4)$$

V_o: initial velocity of an enzyme inhibited reaction, V_{max}: maximum reaction rate, K_m: Michaelis constant, K_i: the dissociation constant for the enzyme-inhibitor complex, K_i': the dissociation constant for the enzyme substrate-inhibitor complex, S: concentration of substrate.

Determination of antioxidant activities

ABTS radical scavenging activity

This experiment was conducted in accordance with Gaber *et al.* (2021).[15] The radical cation of 2,2′-azino-bis-ethylbenzthiazoline-6-sulfonic acid ($ABTS^+$) was produced by combining 7mM ABTS solution with 2.45mM potassium persulfate at a 1:1 (v/v) ratio and leaving the mixture for 4 to 16 hours until a stable absorbance was achieved. The $ABTS^+$ solution was diluted with ethanol to achieve an absorbance of 0.70 ± 0.05 at 734nm. The photometric experiment was carried out by combining $ABTS^+$ solution and crude extract at various concentrations for 45 seconds.

After one minute of incubation in the dark, the absorbance mixture was measured at 734nm. The blank was the sample's solvent solution, the negative control was solvent with $ABTS^+$, and the positive control was butylated hydroxytoluene (BHT) with $ABTS^+$. All tests were performed at least three times. The formula for calculating the percentage of radical inhibition is as follows:

$$\text{Inhibition (\%)} = \frac{(A\,control - A\,sample)}{A\,control}\text{x}100 \qquad (5)$$

A control was the absorbance of the ABTS+ solution without extract, and A sample was the absorbance of the sample with ABTS+. The half-maximal inhibitory concentration (IC_{50}) was defined as the amount of antioxidant required to reduce the initial $ABTS^+$ concentration by 50%. The blank was the sample's solvent solution, the negative control was solvent with ABTS+, and the positive control was butylated hydroxytoluene (BHT) with $ABTS^+$. All tests were performed at least three times.

DPPH radical scavenging activity

The radical scavenging activity of the sample was determined using the DPPH technique, as described by Ekasari et al. (2023).[16] The crude extract was serially diluted and combined with 0.3mM DPPH solution before being incubated in the dark at room temperature for 20 minutes.

A spectrophotometer was used to evaluate the decrease in absorbance of the sample relative to the DPPH and methanol mixture (control) at 517nm. The antioxidant activity of the samples was calculated by comparing them to the DPPH solution at the same concentration using the ABTS equation.

Assessment of anti-colorectal cancer activities

Cell lines preparation and culture

This study using two types of colorectal cancer cell lines, which are HCT116 and SW620 cell lines. The cells were initially grown in Gibco Dulbecco's Modified Eagle Medium (DMEM). The two cells were then grown at 37°C in a humid environment containing 5% CO_2. The cells went through two passages before being used in the experiment.

Cytotoxic activity

The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was performed to determine the percentage of cell viability. The following method was used to cultivate $5x10^3$ cells/well in a 96-well plate for 24 hours, fresh media was added with varying concentrations of the extract (0-1000µg/ml), and incubated for 48 hours. The absorbance was measured using an ELISA reader at 570nm. The negative control was an untreated cell with DMSO, 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 obtained data was utilized to calculate the percentage of viable/dead cells using the equation[17]

$$\text{cell viability (\%)} = \frac{Abs\ of\ sample}{Abs\ of\ sample} \text{ x } 100 \quad (6)$$

Abs of the sample was the absorbance of treated cells, and Abs of control was the absorbance of untreated cells. The half-maximal inhibitory concentration (IC_{50}) was defined as the ability of a compound concentration to inhibit cell growth by 50%. The IC_{50} was calculated using curves built by graphing cell survival (%) versus sample concentration. The blank was DMSO with MTT solution, the negative control was untreated cells with DMSO and MTT, and the positive control was cisplatin-treated cells with DMSO and MTT [17,18].

Statistical analysis

The values represented the IC_{50}. All tests were performed in at least three replicates. p-values were determined using ANOVA and IBM SPSS; p-values less than 0.05 were considered statistically significant.

Results and Discussion

In this work, the antioxidant, antidiabetic, and anticancer properties of *Coccinia grandis* stems fractions and compounds were examined. Cancer is a serious disease with a high fatality rate. In addition to hereditary risk factors, poor socioeconomic lifestyles have evolved to have a negative impact on cancer's latent risk variables.[1] Furthermore, it has been demonstrated that type 2 diabetes considerably elevates the chance of several cancer conditions, including colorectal cancer. [19]

C. grandis has implications in pharmacological plant research since it has a range of traditional therapeutic applications. Previous research has shown that *C. grandis* had a variety of active compounds and bioactivities that prevent free radicals, cancer, and diabetes. [20,21] The partitioned methanol extract with different polarity solvents; ethyl acetate and n-hexane yielded n-hexane, ethyl acetate, and methanol fractions, which were non-polar, semi-polar, and high-polar, respectively. These fractions were employed in the current study to bring out the detection of *C. grandis* bioactivities.

Bioassay guide of α-glucosidase inhibitory activity

The fractions were screened for rat intestinal α-glucosidase inhibitory activity using maltose and sucrose as substrates. Table 1 results showed that methanol and ethyl acetate fractions have higher percentage inhibition of α-glucosidase than n-hexane extract. Regarding the highest yielded of ethyl acetate fraction obtained; 14.10g compared to 0.80g of methanol fraction, thus the ethyl acetate fraction was performed to gain the identified compounds content. Furthermore, the fraction3 (EA3) of ethyl acetate extracts exhibited the inhibition of α-glucosidase around 30% on both substrates. Then, the further fractions from EA3 also resulted the higher percentage on α-glucosidase inhibition, especially the screening result of EA3.2 (maltase inhibition: 44.68%, sucrose inhibition: 28.84%), with IC_{50} results against maltase was lower (EA3.2: 11.18mg/ml) compared to EA3.1 (483.90mg/ml) and IC_{50} of EA3.2 was decreased against sucrose (19.49mg/ml) EA3.3 (36.45mg/ml) (Table 2).

Table 1: *α*-glucosidase inhibitory activity of *C. grandis* stems fractions using maltose and sucrose as substrates, and acarbose as positive control.

CG stem fraction	% Maltase Inhibition (mg/ml)	% Sucrase Inhibition (mg/ml)
n-hexane	15.77	7.93
Ethyl acetate (EA)	22.39	30.17
Methanol	43.39	29.16
EA1	N/A	N/A
EA2	8.97	2.27
EA3	30.21	32.48
EA4	17.19	10.35
EA3.1	6.27	0.06
EA3.2	44.68	28.84
EA3.3	25.8	3.88
Acarbose	96.81	88.35

Characteristics of the compounds isolated from C. grandis stems fraction.

The further analysis for compound identification was conducted through the EA3 fraction, which contained seven compounds (Figure 1): vanillic acid, syringaldehyde, 4-hydroxybenzaldehyde, ferulic acid, p-hydroxycoumaric acid, methyl caffeate and caffeic acid. Figure 1 showed chemical structure of compounds 1-7.

Compound 1 (Vanillic acid) : pale-yellow powder; ^{1}H NMR)$CDCl_3$, 400 MHz :(δ 3.83) 3H, *brs*, OCH_3(, 7.25)1H, d, J=8.0 Hz(, 7.43) 1H, *brs*(, 7.61)1H, d, J=8.0 Hz(, 9.55) 1H,-OH(, 12.74)1H, COOH(, ^{13}C NMR)$CDCl_3$, 100 MHz :(123.8) C-1(, 124.0) C-2(, 114.1) C-3(, 153.9)C-4(, 149.1) C-5(, 114.7)C-6(, 56.1) OCH_3-5(, 169.3 (C=O). Chemical formular $C_8H_8O_4$. Compound 2 (Syringaldehyde) : pale-yellow powder; ^{1}H NMR)$CDCl_3$, 400 MHz :(δ 3.86) 6H, *brs*, 3,5- OCH_3(, 7.06)2H, *brs*(, 9.68) 1H,CHO(^{13}C NMR)$CDCl_3$, 100 MHz :(128.0) C-1(, 106.7)C-2,6(, 147.4) C-3,5(, 141.1) C-4(, 56.3) OCH_3-3,5(, 191.0 (CHO). Chemical formula $C_9H_{10}O_4$.

Table 2: IC_{50} value of *α*-glucosidase inhibitory of the fractions from ethyl acetate fraction of *C. grandis* stem using maltose and sucrose as substrates, and acarbose as positive control

CG stem fraction	IC_{50} (mg/ml) against maltase	IC_{50} (mg/ml) against sucrose
Ethyl acetate 3(EA 3)	13.552	9.728
EA 3.1	483.90	15.86
EA 3.2	11.18	19.49
EA 3.3	9.53	36.45
EA 3.2.1	25.80	16.33
EA 3.2.2	8.27	81.61
EA 3.2.3	8.02	6.79
EA 3.2.4	4.31	24.81
EA 3.2.5	21.89	7.15
Acarbose	0.32	0.77

Compound 3 (4-hydroxyBenzaldehyde) : pale-yellow powder; ^{1}H NMR)$CDCl_3$, 400 MHz :(δ 6.83) 2H, dd, J=6.8, 2.0 Hz, 3, 5(, 7.67)2H, dd, J=6.8, 2.0 Hz, 2, 6(, 9.70) 1H,CHO(^{13}C NMR)$CDCl_3$, 100 MHz :(128.9) C-1(, 132.3) C-2,6(, 115.8) C-3,5(, 162.9) C-4(, 191.2 (CHO). Chemical formular $C_7H_6O_2$.

Compound 4 (Ferulic acid) : pale-yellow powder; ^{1}H-NMR)$CDCl_3$, 400 MHz :(3.92) 3H, brs, 3′-OCH_3(, 6.27) 1H, d, J=14.0 Hz, H-2(, 6.91)1H, d, J=8.0 Hz, H-6′(, 7.08) 1H, d, J=8.0 Hz, H-5′(, 7.03) 1H, s, H-2′(, 7.68) 1H, d, J=14.0 Hz, H-1). ^{13}C-NMR)$CDCl_3$, 100 MHz :(114.2)C-1(, 146.9)C-2(, 126.7)C-1′(, 109.4)C-2′(, 151.9)C-3′(, 146.9)C-4′(, 114.8)C-5′(, 123.5)C-6′(, 55.9)3′-OCH_3(, 172.5)C=O(. Chemical formular $C_{10}H_{10}O_4$.

Compound 5 (*p*-Hydroxy Coumaric acid) : pale-yellow powder; ^{1}H-NMR)$CDCl_3$, 400 MHz :(6.18) 1H, dd, J=16.0, J=8.0 Hz, H-2(, 6.78)2H, dt, J=8.0, 1.6 Hz, H-3′, 5′(, 7.33) 2H, dt, J=8.0, 1.6 Hz, H-2′, 6′(, 7.56) 1H, dd, J=16.0, J=8.0 Hz, H-1(, 7.30) 1H, d, J=8.0 Hz, 4′-OH(. ^{13}C-NMR)$CDCl_3$, 100 MHz :(114.5)C-1(, 145.5)C-2(, 126.0)C-1′(, 129.9) C-2′(, 115.7)C-3′(, 159.7)C-4′(, 115.7)C-5′(, 129.9)C-6′(, 171.5)C=O(. Chemical formular $C_9H_8O_3$.

Compound 6 (Methyl Caffeate) : pale-yellow powder; ^{1}H-NMR)$CDCl_3$, 400 MHz :(3.71) 3H, brs, -OCH_3(, 6.16) 1H, d, J=16.0 Hz, H-1(, 6.75) 1H, d, J=7.0 Hz, H-6′(, 6.86) 1H, d, J=7.0 Hz, H-5′(, 6.98) 1H, brs, H-2′(, 7.49) 1H, d, J=16.0 Hz, H-2(. ^{13}C-NMR)$CDCl_3$, 100 MHz :(113.8)C-1(, 144.6)C-2(, 126.8)C-1′(, 114.1)C-2′(, 145.4)C-3′(, 147.2)C-4′(, 115.1)C-5′(, 121.9)C-6′(, 51.5)-OCH_3(, 168.3)C=O(. Chemical formular $C_{10}H_{10}O_4$.

Compound 7 (Caffeic acid) : pale-yellow powder; ^{1}H-NMR)$CDCl_3$, 400 MHz :(6.27) 1H, d, J=16.0 Hz, H-1(, 6.67) 1H, d, J=7.0 Hz, H-6′(, 6.82) 1H, d, J=7.0 Hz, H-5′(, 7.02) 1H, brs, H-2′(, 7.45) 1H, d, J=16.0 Hz, H-2(. ^{13}C-NMR)$CDCl_3$, 100 MHz :(116.5)C-1(, 144.9)C-2(, 128.0)C-1′(, 115.2)C-2′(, 145.9)C-3′(, 146.5)C-4′(, 117.2)C-5′(, 123.2)C-6′(, 171.5)C=O(. Chemical formular $C_9H_8O_4$.

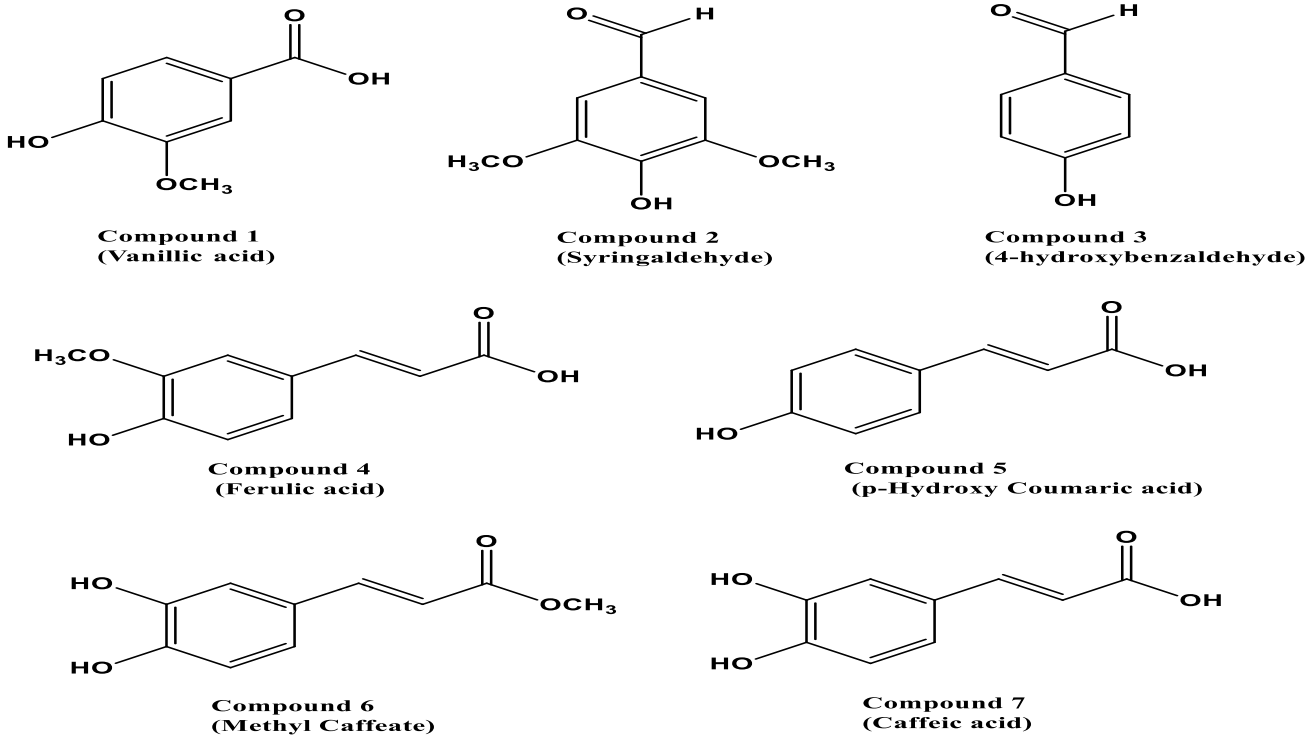

Figure 1: The structure of identified compounds 1-7

Assessment of antidiabetes activities with rat intestinal the α-glucosidase inhibition

The Antidiabetic using rat intestinal α-glucosidase inhibitory activity of Coccinia grandis stems fractions

Table 3 stated the inhibitory properties of *C. grandis* stem fractions on rat *α*-glucosidase enzyme activity with sucrose and maltose substrates. The experiment using maltose as a substrate for inducing maltase found that the methanol fraction had the best inhibitory activity with the lowest IC_{50} (6.511 mg/ml), followed by the n-hexane fraction (9.924 mg/ml) and the ethyl acetate fraction (13.552 mg/ml). When sucrose was used as a substrate to produce sucrase, the methanol fraction showed the best inhibitory activity with the lowest IC_{50} (9.175 mg/ml), followed by the ethyl acetate fraction (9.728 mg/ml) and the n-hexane fraction (24.929 mg/ml). Due to delaying the absorption of glucose by inhibiting digestive organ enzymes like *α*-glucosidase is one therapy strategy for diabetes, and the rate of hydrolytic cleavage of oligosaccharides slows when intestinal *α*-glucosidase is suppressed.[22,23] The study concluded that polar fractions were effective via inhibiting *α*-glucosidase.

Table 3: *α*-glucosidase inhibitory activities of *Coccinia grandis* stems fractions

Fractions	IC_{50} against maltase (mg/mL)	IC_{50} against sucrase (mg/mL)
n-hexane fraction	9.924 ±0.400[b]	24.929±0.493[c]
ethyl acetate fraction	13.552 ±0.727[c]	9.728±0.273[b]
methanol fraction	6.511 ±0.242[a]	9.175±0.317[a]
Acarbose as positive control	0.064 ±0.003 mM	0.244±0.003 mM

One-way ANOVA with Duncan test, p-value<0.05. The subscribed letter indicated the significant value between fractions in each group maltase substrate or sucrose substrate. Acarbose as positive control.

The Antidiabetic using rat intestinal α-glucosidase inhibitory activity of Coccinia grandis stems isolated compounds

Table 4 indicated the inhibitory actions of *C. grandis* stems compounds against rat *α*-glucosidase enzyme activity. The experiment using maltose as a substrate for inducing maltase revealed that caffeic acid had the highest inhibitory activity with the lowest IC_{50} (0.732 mM), followed by methyl caffeate (0.838 mM), ferulic acid (1.035 mM), p-hydroxycoumaric acid (19.034 mM), syringaldehyde (36.992 mM), vanillic acid (53.228 mM), and 4-hydroxybenzaldehyde (300.455 mM), respectively. Caffeic acid had the highest inhibitory effectiveness with the lowest IC_{50} (0.111 mM) in the experiment using sucrose as a substrate to generate sucrase, followed by methyl caffeate (0.114 mM), ferulic acid (0.421 mM), p-hydroxy coumaric acid (1.136 mM), syringaldehyde (19.285 mM), vanillic acid (53.228 mM), and 4-hydroxybenzaldehyde (103.508 mM), respectively.

Table 4: *α*-glucosidase inhibitory activities of *Coccinia grandis* stems identified compounds

Fractions	**IC_{50} against maltase (mM)**	**IC_{50} against sucrase (mM)**
Caffeic acid	0.732±0.059[a]	0.111±0.016[a]
p-Hydroxy Coumaric acid	19.034±0.019[d]	1.136±0.065[c]
Ferulic acid	1.036±0.149[c]	0.422±0.026[b]
4-hydroxyBenzaldehyde	300.455±0.028[g]	103.509±0.179[f]
Methyl Caffeate	0.838±0.059[b]	0.114±0.015[a]
Syringaldehyde	36.992±0.088[e]	19.286±0.051[d]
Vanillic acid	53.229±0.070[f]	33.671±0.042[e]
Acarbose as positive control	0.064±0.003	0.244±0.003

One-way ANOVA with Duncan test, p-value<0.05. The subscribed letter indicated the significant value between fractions in each group maltase substrate or sucrose substrate. Acarbose as positive control.

Evaluation of the nature mechanism of the α-glucosidase inhibition

The previous inhibitory mechanisms investigation of caffeic acid using alpha-glucosidase enzyme isolated from yeast and used pNPG as a substrate, the compound was studied using Lineweaver-Burk and identified *α*-glucosidase inhibitor, the kinetic parameters showed that caffeic acid is mixed competitive inhibition types. [22] Although methyl caffeate displayed inhibition against rat intestinal *α*-glucosidase, scientific proof and reports of this compounds related inhibitory effects have not yet been established. To acquire a better understanding of how methyl caffeate interacts with rat intestinal maltase and sucrase, its inhibitory method was investigated using a kinetic research. A line weaver-Burk plot against maltase (Figure 2) and sucrase (Figure 3) revealed a sequence of straight lines. The junction of the straight lines versus maltase (Figure 2) was on the x-axis, indicating that methyl caffeate retarded maltase function in a competitive manner which occurs when the inhibitor binds to the enzyme's active site, preventing the substrate from binding. Furthermore, the straight lines against sucrase (Figure 3) were parallel; methyl caffeate retarded sucrase in an uncompetitive manner, on the other hand, occurs when the inhibitor binds to the enzyme-substrate complex, altering the reaction rate. Due to its potential bioactivities, the mechanism of inhibition of methyl caffeate of *α*-glucosidase against rat intestinal cells was investigated.[24]

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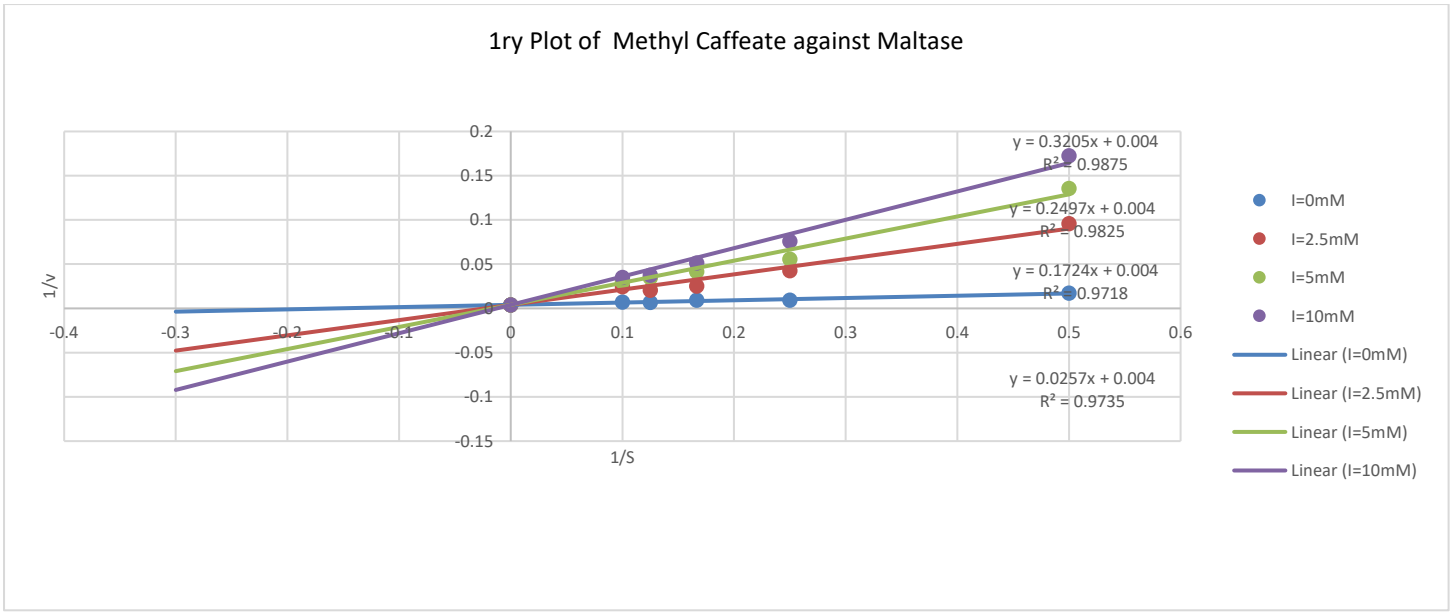

Figure 2: The kinetic investigation of methyl caffeate indicated that it retarded maltase function in a competitive manner.

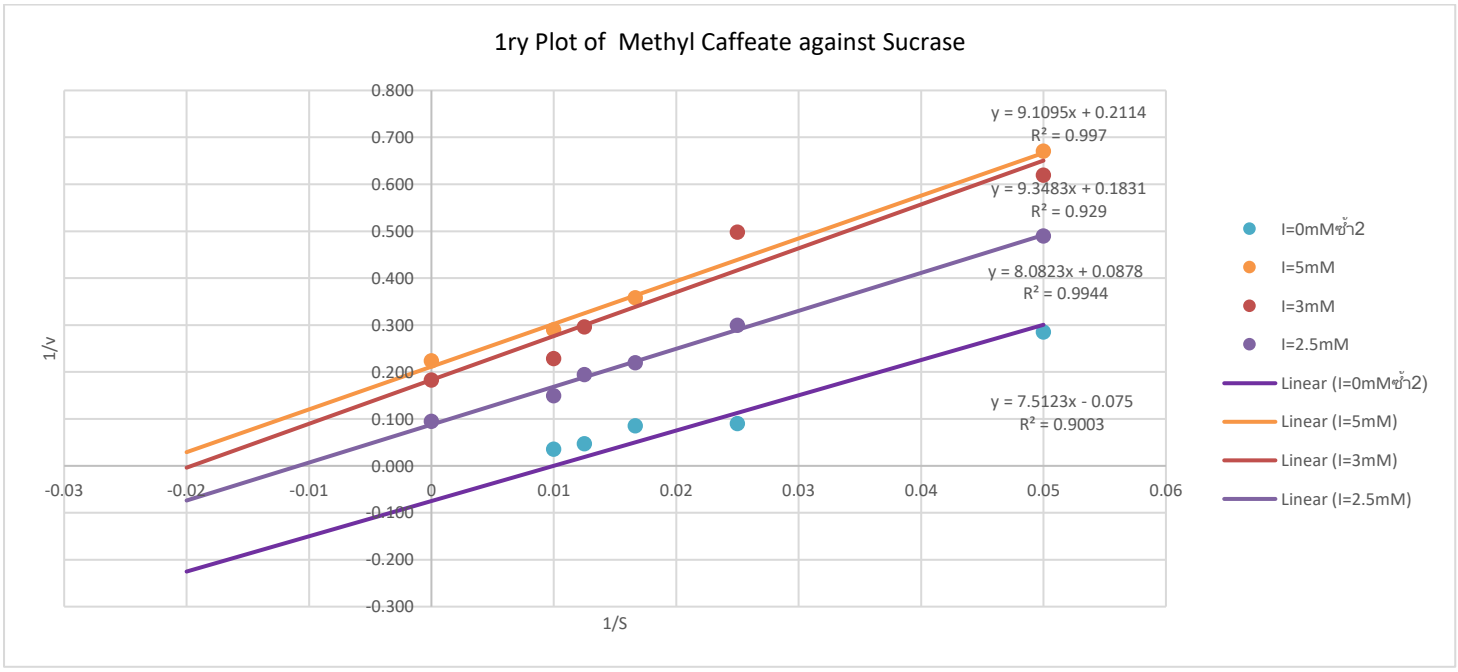

Figure 3: The kinetic investigation of methyl caffeate indicated that it is against sucrase function in an uncompetitive manner.

Determination of antioxidant activities

Antioxidant activities using DPPH and ABTS reagents of Coccinia grandis stems fractions

Table 5 presented the antioxidant activity of *C. grandis* fraction samples against free radical reagents, as well as the samples' DPPH and ABTS scavenging activity. In terms of half maximal inhibitory concentration (IC_{50}) results, DPPH results demonstrated that ethyl acetate fraction showed the highest scavenging ability with the lowest IC_{50} (0.852 mg/mL), followed by n-hexane fraction (19.953 mg/mL) and methanol fraction (19.954 mg/mL); however, the n-hexane fraction and methanol fraction did not show significant differences in results. Similarly, ABTS results revealed that the ethyl acetate fraction had the strongest scavenging ability with the lowest IC_{50} (0.373 mg/mL), followed by the n-hexane fraction (26.445 mg/mL) and the methanol fraction (28.196 mg/mL), respectively, with significant differences.

The antioxidant characteristics assay showed that the ethyl acetate fraction had significantly higher DPPH and ABTS scavenging activity ($p<0.05$) (Table 5). While the ABTS data showed that the n-hexane fraction had higher activity than methanol fraction, there was no difference in DPPH scavenging activity. It can be inferred that semi-polar fractions which was contained with semi-polar phytoconstituents have higher potential antioxidant properties than polar and non-polar solvents. Thus, it was proposed that the ethyl acetate fraction of *C. grandis* stems may be developed as a viable antioxidant agent. Another study found that antioxidant capabilities are beneficial in reducing non-communicable diseases (NCDs), including diabetes and cancer. An increase in free radical generation, as well as a decrease in antioxidant defense, may all contribute to the beginning and progression of diabetic complications. Chronic hyperglycemia in diabetic patients is a major cause of oxidative stress due to excessive ROS generation, which may contribute to a variety of diabetic complications such as damaged cells and tissues.[25]

Table 5: Antioxidant activities of *Coccinia grandis* stems fractions

Fractions	IC_{50} of DPPH (mg/mL)	IC_{50} of ABTS (mg/mL)
n-hexane	19.953± 0.732 [b]	26.445±0.072[b]
Ethyl acetate	0.852±0.118[a]	0.373±0.135[a]
Methanol	19.954±0.713[b]	28.196±0.112[c]
BHT as positive control	0.537± 0.005 mM	0.901±0.001 mM

One-way ANOVA with Duncan test, p-value<0.05. The subscribed letter indicated the significant value between fractions in each group DPPH or ABTS. BHT: butylated hydroxyl-toluene as positive control.

Antioxidant activities using DPPH and ABTS reagents of Coccinia grandis stems isolated compounds

Table 6 demonstrated the antioxidant properties of *C. grandis* stems compound samples on free radical reagents. In terms of half maximal inhibitory concentration (IC_{50}) results, DPPH results demonstrated that methyl caffeate showed the highest scavenging ability with the lowest IC_{50} (0.145 mM), followed by caffeic acid (0.154 mM), p-hydroxy coumaric acid (0.535 mM), ferulic acid (1.043 mM), syringaldehyde (1.744 mM), and vanillic acid (6.665 mM), respectively. Ferulic acid had the highest scavenging ability with the lowest IC_{50} (0.057 mM), followed by methyl caffeate (0.143 mM), p-hydroxy coumaric acid (0.173 mM), vanillic acid (0.185 mM), caffeic acid (0.194 mM), syringaldehyde (1.587 mM), and 4-hydroxybenzaldehyde (58.527 mM), respectively.

Table 6: Antioxidant activities of *Coccinia grandis* stems identified compounds

Fractions	IC_{50} of DPPH (mM)	IC_{50} of ABTS (mM)
Caffeic acid	0.154±0.044[b]	0.194±0.015[e]
p-Hydroxy Coumaric acid	0.535±0.015[c]	0.173±0.008[c]
Ferulic acid	1.043±0.002[d]	0.057±0.018[a]
4-hydroxyBenzaldehyde	59.172±0.038[g]	58.527±0.021[g]
Methyl Caffeate	0.145±0.014[a]	0.143±0.027[b]
Syringaldehyde	1.744±0.011[e]	1.587±0.025[f]
Vanillic acid	6.665±0.002[f]	0.185±0.063[d]
BHT as positive control	0.537± 0.005 mM	0.901±0.001 mM

One-way ANOVA with Duncan test, p-value<0.05. The subscribed letter indicated the significant value between fractions in each group DPPH or ABTS. BHT: butylated hydroxyl-toluene as positive control.

Assessment of anti-cancer activities

Anticancer activities on colorectal cancer cell lines of Coccinia grandis stems fractions

Table 7 showed anti-cancer activities of *Coccinia grandis stems fractions* against HCT116 and SW620 colorectal cell lines. The results showed that the methanol fraction did not inhibit HCT116 and SW620 cell viability. The n-hexane fraction exhibited the most significant reduction in HCT116 and SW620 cell viability, with the lowest IC_{50} (18.159 µg/ml, 22.395 µg/ml, respectively), followed by the Ethyl acetate (26.135 µg/ml, 84.053 µg/ml, respectively).

It was indicated that the non-polar and semi-polar fractions absorbed more bioactive chemicals as anti-colorectal cancer agents than the polar solvents.

Table 7: Anti-colorectal cancer activities of *Coccinia grandis* stems fractions

Fractions	IC_{50} against HCT116 (µg/mL)	IC_{50} against SW620 (µg/mL)
n-hexane	18.159±0.045[a]	22.395±[a]
Ethyl acetate	26.135±0.123[b]	84.053±[b]
Methanol	ND	ND
Cisplatin as positive control	0.003±0.001 mM	0.008±0.001 mM

One-way ANOVA with Duncan test, p-value<0.05. The subscribed letter indicated the significant value between fractions in each group HCT116 or SW620.

ND: non detectable. Cisplatin as positive control.

Anticancer activities on colorectal cancer cell lines of C. grandis stems isolated compounds

Table 8 revealed the anti-colon cancer activity of *C. grandis* stems compounds, as well as the decrease in cell viability of HCT116 and SW620 cell lines. The IC_{50} values for each treatment were used to assess the cytotoxicity of the drugs. The IC_{50} values obtained in the experiment in which the HCT116 and SW620 cell lines were treated with compounds derived from C. grandis stems and cisplatin are displayed in dose-inhibition mode. Methyl caffeate had the lowest IC_{50} (0.001 mM) for inhibiting cell viability in HCT116, followed by caffeic acid (0.012 mM), syringaldehyde (0.188 mM), vanillic acid (0.686 mM), p-hydroxy coumaric acid (0.782 mM), ferulic acid (0.977 mM), and 4-hydroxybenzaldehyde (1.346 mM). Caffeic acid had the lowest IC_{50} (0.085 mM) for inhibiting cell viability in SW620, followed by methyl caffeate (0.165 mM), ferulic acid (0.323 mM), syringaldehyde (0.382 mM), p-hydroxy coumaric acid (0.449 mM), 4-hydroxybenzaldehyde (0.588 mM), and vanillic acid (1.483 mM).

The results imply that each bioactivity assay would react differently to the fractions due to the unique active chemicals they contained. For these reasons, based on the bioactivities results, polarity, and quantity, the ethyl acetate fraction was further examined, which included comprehensive phytochemical identification. The ethyl acetate fraction

was isolated, purified, and described, yielding seven compounds: caffeic acid, p-hydroxycoumaric acid, ferulic acid, 4-hydroxybenzaldehyde, methyl caffeate, syringaldehyde, and vanillic acid.

These *C. grandis* compounds were tested for antioxidant, antidiabetic, and anti-colorectal cancer activity. These activities produced interesting results, with methyl caffeate showing the best antioxidant properties against DPPH, as well as antidiabetic properties against sucrase, and anti-colorectal cancer properties against HCT116. While caffeic acid had the most anti-diabetic effects against maltase and sucrase, it also had significant anti-colorectal cancer activity against SW620. Caffeic acid is a naturally occurring phenolic compound, and methyl caffeate is an ester of it. Methyl caffeate, an α-glucosidase inhibitor, was identified as a rat intestine sucrase and maltase inhibitor using enzyme-assay guided fractionation of *Solanum torvum* fruit extract. The oxidative dimerization product of methyl caffeate is regarded to be a promising oncoprotective, potentially preventing cancer formation and spread. It can also limit the proliferation of breast cancer cells at a level that is suitable for pharmaceutical usage. [26,27] Methyl caffeate had the greatest findings in this investigation; it was a strong antioxidant against DPPH and ABTS, had a considerable anti-colorectal cancer impact against HCT116 and SW620, and had the second highest antidiabetic effect against sucrose and maltase. Ferulic acid, a common phenolic compound, has a variety of biological activities, including hepatoprotective, antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and antioxidant activity. It also influences the activity of enzymes.[28] The findings of this study revealed that ferulic acid had a wide range of effects, including being the best antioxidant against ABTS but moderate against DPPH, ranking second against sucrase and third against maltase, and having a moderate anticancer effect against SW620 but appearing to be low against HCT116. Similar to BHA, BHT, a-tocopherol, and ascorbic acid, p-coumaric acid, also known as 4-hydroxycinnamic acid, is a naturally occurring phenolic compound having antibacterial, anti-inflammatory, and antioxidant properties, as well as the potential to scavenge free radicals. In comparison to the other bioactivities in this investigation, p-hydroxycoumaric acid had a moderate degree of each.[29] Syringaldehyde, a significant odorant in whisky and brandy, has medicinal properties related to antioxidants, inflammation, diabetes, and tumor necrosis factor. SA performed moderately on all investigations in the research findings.[30] Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is a naturally occurring aromatic phenolic acid present in many herbs and used as a flavoring ingredient. Numerous studies have demonstrated its different pharmacological properties, including anti-venom, anti-inflammatory, antibacterial, cardioprotective, hepatoprotective, free radical scavenging, and antioxidant activity. However, comparing vanillic acid to the other compounds in each bioactivity in this investigation produced unexpected outcomes.[31] 4-hydroxybenzaldehyde (4-HBA), 4-HBA and 3-HBA were created a protective phenotype by inhibiting apoptosis when *Angiostrongylus cantonensis* young adult ESPs were treated. Thus, 3-HBA and 4-HBA are prospective therapeutic drugs for the treatment of human angiostrongyliasis.[32] However, comparing 4-HBA to the other compounds in each bioactivity yielded unexpected outcomes.

Previous research has shown that plant type, planting area, crop age, post-harvest treatment, extraction method, and extract solvent all have an impact on bioactive chemicals obtained by extraction. A number of characteristics, including solvent type, extraction method, and sample type. As an example, a research of natural products obtained from higher plant species, *Boesenbergia rotundo* Linn., discovered that the ethanol fraction, a high polar fraction, had much higher potential antifungal activity than the dichloromethane fraction, a semi-polar fraction. [33] In a similar case, a study comparing the effectiveness of Zingiberaceae family species found that the dichloromethane fraction, or semi-polar fraction, from galangal inhibited *Rhizoctonia solani* Kuhn, a pathogenic fungus, significantly more than the methanol fraction, which is a high polar fraction.[34] As a result, the varied polarities of solvent extraction are expected to yield diverse biocompounds. [35]

This study found that different fractionated polarity solvents yielded diverse findings in bioactivity measurements. As a result, developing a desirable solvent is an interesting prospect for obtaining the most efficient bioactive chemical for certain bioactivity requirements. Consuming extracts obtained from simple processes using polarity-based fractionation is preferable to obtain treatment that fulfills the intended purpose.

Table 8: Anti-colorectal cancer activities of *Coccinia grandis* stems identified compounds

Fractions	**IC_{50} against HCT116 (mM)**	**IC_{50} against SW620 (mM)**
Caffeic acid	0.012 ± 0.009^{b}	0.085 ± 0.003^{a}
p-Hydroxy Coumaric acid	0.782 ± 0.004^{e}	0.449 ± 0.004^{e}
Ferulic acid	0.977 ± 0.001^{f}	0.323 ± 0.001^{c}
4-hydroxyBenzaldehyde	1.346 ± 0.010^{g}	0.588 ± 0.024^{f}
Methyl Caffeate	0.001 ± 0.0006^{a}	0.165 ± 0.001^{b}
Syringaldehyde	0.189 ± 0.005^{c}	0.382 ± 0.002^{d}
Vanillic acid	0.687 ± 0.001^{d}	1.483 ± 0.006^{g}
Cisplatin as positive control	0.003±0.001 mM	0.008±0.001 mM

One-way ANOVA with Duncan test, p-value<0.05. The subscribed letter indicated the significant value between fractions in each group HCT116 or SW620. ND: non detectable. Cisplatin as positive control.

Conclusion

This present study demonstrated that some different fractions and some purified compounds of *C. grandis* stems have α-glucosidase inhibitory, antioxidant and anti-colorectal cancer abilities in the different manner. Furthermore, the findings of this study indicated that the stems of *C. grandis* contain bioactive compounds, and could develop to serve as drug candidates.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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