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Original Research Article

Phytochemical Screening and Antioxidant Activities of *Cornukaempferia chayanii* Extracts and *in vitro* Inhibitory Effects on Digestive Enzymes

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ABSTRACT

Cornukaempferia chayanii is a new species of plant from the genus *Cornukaempferia* belonging to the family Zingiberaceae. The plant is an endemic species used as a folk medicine in northern and northeastern Thailand. The present study was carried out to conduct phytochemical screening, assess antioxidant activity, and determine the *in vitro* inhibitory effects of *C. chayanii* extracts on *α*-glucosidase and *α*-amylase enzymes. *C. chayanii* was collected, processed, and extracted with aqueous (CCA), 50% ethanol (CCHE), and 95% ethanol (CCE). A preliminary phytochemical screening was performed to determine the total phenolic (TPC) and flavonoid (TFC) contents. The antioxidant activities of the extracts were examined by DPPH (2,2-diphenyl-1-picrylhydrazy), $ABTS^+$ (2,2-azinobis-[3-ethylbenzothiazoline-6-sulphonate]) radical scavenging, and FRAP (ferric reducing antioxidant power) assays. The *in vitro* inhibitory activities of the extracts on α-glucosidase and *α*-amylase enzymes were also evaluated. The percentage yields of CCA, CCHE, and CCE were 13.22±0.54%, 8.13±0.12%, and 5.02±0.57%, respectively. CCHE had much higher TPC than the CCA and CCE, while TFC was more in the CCE than the other extracts. According to the DPPH and FRAP assays, the CCHE demonstrated significantly higher free radical scavenging activity than the other extracts. Moreover, the CCHE had a similar effect compared to ascorbic acid and Trolox (reference compounds) with the DPPH assay. The CCA had a significant *in vitro* α-glucosidase and α-amylase enzyme inhibitory effect more than Acarbose, a standard diabetic drug. The findings of this study show that *C. chayanii* has antioxidant, α-glucosidase, and α-amylase inhibitory properties, and as such, may benefit human health.

Keywords: Antioxidation, Flavonoids, Phenolic compounds, α-glucosidase, α-amylase, *Cornukaempferia chayanii*.

Introduction

Flavonoids are a type of secondary metabolite of phenolic compounds found most abundantly in plants. It contains 2-aromatic rings, carbonyl groups, and hydroxyl groups. These chemicals are produced by plants to protect them from biotic and abiotic stressors.[1] Furthermore, they are synthesized to fight predators and microorganisms based on their poisonous nature and to repel bacteria and herbivores.[2] Antioxidation, anti-diabetes, anti-hypertension, and health promotion are among the benefits of natural products derived mostly from herbal plants.[3] The uncontrolled synthesis and accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have resulted in a wide range of human disorders. Antioxidant compounds are chemical components found in plants that can protect or mitigate damage to organs.[4] Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), glutathione S-transferase (GST), and glutathione (GSH) are antioxidant substances that prevent and maintain the balance between the production and neutralization of ROS in the human body.[5]

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The α-amylase and α-glucosidase enzymes are digestive enzymes that break down carbohydrates into smaller sugars known as glucose, which are then converted to individual basic glucose units and absorbed in the blood vessel system.[6] Inhibitors of the α-amylase and α-glucosidase enzymes would limit the digestive system, resulting in lower sugar levels, which would minimize obesity, diabetes, and other disorders.[7]

Thailand is regarded as one of the world's most resource-rich countries. The topographical and climatic conditions in this area are ideal for the survival and growth of tropical dry or deciduous forests. New species of plants are constantly being discovered around the world, and several technical items can be turned into medicine, food, ornamental plants, and other products that are beneficial to humanity.[8] *Cornukaempferia chayanii* is a new species belonging to the Zingiberaceae family. The genus is endemic to northern and northeastern Thailand. The plant is characterized by its yellowish to the orange-yellow rhizome, short pseudostem with 2-3 broad leaves, prostrate or nearly to the ground, inflorescence with 2-15 flowers, and a yellow flower with long, narrow, front, and curved anther crest. The yellow flowers start opening in the early evening. The plant can be found in both dry dipterocarp and mixed deciduous forests.[9] The genus of this plant has been used by the locals to treat hemorrhoids and laryngitis in Thai children.[10] Although, *C. chayanii* is a recently discovered species, no scientific study has proven its use.

The present study was aimed at conducting a phytochemical screening and evaluating the antioxidant activities of *C. chayanii* extracts. Also, the *in vitro* inhibitory effects on α-glucosidase and α-amylase enzymes were investigated.

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Materials and Methods

Source and identification of the plant

Cornukaempferia chayanii was collected in Uttaradit province, Thailand, in May-June 2017. The specimen was identified (ID Code: Yupparach & Wongsuwan 120616-1) and deposited at the Faculty of Medicine, Mahasarakham University, Thailand. The plant material was cleaned and dried in a hot air oven at 60°C for 48 hours before being processed into a fine powder.

Preparation of Cornukaempferia chayanii extracts

The aqueous extract of the plant was prepared by boiling 100 g of plant material twice in 1 L of distilled water for 10 min. The hydro-ethanolic and ethanolic extracts were macerated for 7 days with 50 and 95% ethanol, respectively (100 g per 400 mL). Filter papers (Whatman, Germany) were used to remove the remaining powder. The filtrate was evaporated using a rotary evaporator (Heidolph Laborota 4000, Germany) and freeze-dried to obtain dark brown extracts, which were stored at -20°C until they were needed.

Total phenolic content assay

Total phenolic content was determined according to a modified procedure.[11] One hundred microliters (100 µL) of the sample extract were oxidized with 500 µL of 0.2 N Folin-Ciocalteu's reagent and neutralized by adding 400 µL of 7.5% Na_2CO_3. After mixing and incubating at room temperature for 30 minutes, the absorbance was measured at 765 nm. The results were expressed as gallic acid equivalents (mgGE/g extract).

Total flavonoid content assay

Total flavonoid content (TFC) was estimated using the aluminum chloride colorimetric method.[11] The plant extract was combined with 100 L of 5% aluminum chloride (w/v) and 400 µL of 2.5% Na_2NO_3. After 5 min, 500 µL of 5% $AlCl_3$ was added. The solution was allowed to sit at room temperature for 10 min and then mixed with 2 mL of distilled water. Absorbance values were measured at a wavelength of 415 nm. The TFC was calculated from a standard quercetin equivalent (mgQE/gExt).

Antioxidant activity assays

DPPH radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity of wheat extract was estimated by the reduction of the reaction color between DPPH solution and sample extracts as previously described.[12] DPPH was dissolved in ethanol to 0.039 mg/mL. The plant extract at various concentrations was diluted with distilled water to make test sample solutions. One hundred microliters (100 µL) of the test sample solution were mixed with 900 µL of DPPH (0.1 mM) working solution. After a 30-min reaction, in the dark at room temperature, the absorbance of the solution was measured at 515 nm. Trolox and ascorbic acid were used as standards, while blanks were also run in each assay. The DPPH radical ability was expressed as IC_{50} (mg/mL) and the inhibition percentage was calculated using the following formula:

% radical scavenging activity of DPPH = [$(A_0-A_1)/ A_0$] x 100

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

$ABTS^+$ radical scavenging assay

In the ABTS assay, the plant extract was allowed to react with $ABTS^+$ (2,2-azinobis [3-ethylvenzothiazolin-6-sulphonic acid], a model stable free radical in the assay. The $ABTS^+$ (900 µL) was added to the extract (100 µL) and thoroughly mixed. The solution was kept at room temperature for 6 min before measuring the absorbance at 734 nm. Trolox and ascorbic acid solutions (in 80% ethanol) were prepared and assayed under the same conditions. ABTS scavenging ability was expressed as IC_{50} (mg/mL) and the inhibition percentage was calculated using the following formula:

% radical scavenging activity of ABTS = $[(A_0-A_1)/ A_0] \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.[12]

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity of the medicinal plant was estimated spectrophotometrically following the procedure described by Puangpornpitag *et al.*[13] This reaction is monitored by measuring the change in absorbance at 593 nm. The ferric reducing antioxidant power (FRAP) reagent was prepared by combining 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 proportion at 37°C. Using a 1-5 mL variable micropipette, a freshly made working FRAP reagent was pipetted into 5 µL of adequately diluted plant material and thoroughly mixed. An intense blue color complex was formed when the ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to its ferrous (Fe^{2+}) form and the absorbance at 593 nm was recorded against a reagent blank (3.995 mL FRAP reagent with 5 µL distilled water) after 30 min of incubation at 37°C. The calibration curve was prepared by plotting the absorbance at 593 nm against different concentrations of $FeSO_4$. Also, the concentrations of $FeSO_4$ were plotted against the concentrations of the standard antioxidant, Trolox. The FRAP values were calculated by comparing the change in absorbance in the test solution with those obtained from increasing Fe^{3+} concentrations and were expressed as mg of Trolox equivalent per gram of material.

α-glucosidase inhibitory assay

Using an *in vitro* assay, the extract was evaluated for its ability to inhibit α-glucosidase. The Taepongsorat and Konsue's method was employed with slight modifications.[12] The sample solution (120 µL) was mixed with 100 µL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/mL) and incubated at 37ºC for 20 min. After pre-incubation, 100 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37°C for 20 min. Then, the reaction was stopped by adding 320 µL of 0.2M Na_2CO_3 into each well, and absorbance was measured at 405 nm by a UV-Vis Spectrophotometer, and the values were compared to a control which had 120 µL of buffer solution in place of the extract. Acarbose was used as a positive control, and the system without *α*-glucosidase was used as a blank. The α-glucosidase inhibitory activity was expressed as inhibition (%) and was calculated as follows:

% inhibition of α-glucosidase = $[(A_0–A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. IC_{50} values were calculated by the graphical method.

α-amylase inhibitory assay

The α-amylase inhibitory activity was measured following the standard protocol with slight modifications.[14] Twenty microliters (20 µL) of each extract were combined with 180 L of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 3 units/mL of α-amylase solution (3 units/mL). After incubation at 25°C in a water bath for 10 min, 500 µL of 1% (w/v) starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added, and the solution was incubated at 25°C in a water bath for another 10 min. The reaction was terminated with 500 µL of color reagent (1% w/v of 3,5-dinitrosalicylic acid). The solution was then incubated for 10 min in a boiling water bath. After boiling and cooling to room temperature, 500 L of 40% tartrate solution was added to each tube. The solution was diluted with 100 mL of distilled water. Absorbance was measured at 540 nm with a UV-visible spectrophotometer. The absorbance reading was compared to the control, which included 500 µL of buffer solution, instead of the extract. The α-amylases inhibition percentage was expressed as follows:

% α-amylase inhibition = $[(A_0-A_1)/A_0] \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. IC_{50} values were calculated by the graphical method.

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Statistical analysis

From five separate experiments (n = 5), all the values from the assays were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used in the statistical analysis, followed by Duncan's multiple range tests. Differences at $p < 0.05$ were considered to be significant.

Results and Discussion

The characteristics of *Cornukaempferia chayanii* were botanically described and illustrated in a previous study. The medicinal plant is a new species that was discovered in Southeast Asia, especially Thailand.[9] However, no information on the chemical composition or biological activities has yet been reported. In the present study, the phytochemical composition and biological activities were examined. The extraction process employed produced dark-brown extracts. The percent yield of CCA was much higher than that of CCHE and CCE (Table 1). CCHE had a much higher TPC content than CCA and CCE, as shown in Table 2. Also, TFC was more in the CCE than in the other extracts. The molecular structure of TPC and TFC from natural plants is well known for inhibiting free radicals, which could be beneficial to human health. They were also effective in hypoglycemic activity, which included insulin secretion and insulin resistance.[15] The Thai rural people were encouraged to collect and boil the plant in water for about an hour and then consume the extract as a tonic. They believed the herb could prevent, improve, nourish, and treat a variety of diseases. On the DPPH and FRAP techniques, the CCHE antioxidant activities were significantly more potent. Surprisingly, the CCHE chelate electrons on DPPH, which is similar to the standard substances (ascorbic acid and Trolox) as presented in Table 3. The TPC or/and TFC, which are antioxidant chemicals, maybe the reason for the observation. They donate electrons to free radicals after forming a solid chemical structure.[16] Studies have shown that phenolic compounds, including flavonoids, tannins, and their derivatives, can inhibit free radicals due to their hydroxyl groups at numerous positions.[17] As shown in Table 4, the results revealed that CCA had significantly higher levels of α-glucosidase and α-amylase enzyme inhibition than Acarbose (a diabetic drug). The antidiabetic mechanism might be because of the chemical composition of plants, which contains TPC and/or TFC, which is considered an important target for reducing postprandial hyperglycemia through diet.

Table 1: Percentage yield and color of various extracts from *Cornukaempferia chayanii*

Sample	% yield	colors
CCA	13.22 ± 0.54^a	dark brown
CCHE	8.13 ± 0.12^b	dark brown
CCE	5.02 ± 0.57^c	dark brown

Different letters in each column indicate significant differences at $p < 0.05$; CCA: *Cornukaempferia chayanii* aqueous extract; CCHE: 50% *Cornukaempferia chayanii* ethanolic extract; CCE: 95 % *Cornukaempferia chayanii* ethanolic extract; n = 5.

Table 2: Phytochemical constituents of *Cornukaempferia chayanii* extracts

Sample	TPC (mgGE/gExt)	TFC (mgQE/gExt)
CCA	218.664 ± 5.817^b	110.509 ± 0.652^c
CCHE	323.323 ± 7.853^a	173.652 ± 1.026^b
CCE	125.042 ± 6.687^c	367.221 ± 1.205^a

Different letters in each column indicate significant differences at $p < 0.05$; CCA: *Cornukaempferia chayanii* aqueous extract; CCHE: 50% *Cornukaempferia chayanii* ethanolic extract; CCE: 95 % *Cornukaempferia chayanii* ethanolic extract; n = 5.

Table 3: Antioxidant activities of *Cornukaempferia chayanii* extracts

Sample	DPPH (IC_{50} = mg/mL)	ABTS (IC_{50} = mg/mL)	FRAP (mg = TE/gExt)
CCA	0.654 ± 0.010^c	0.150 ± 0.080^c	9.256 ± 0.567^b
CCHE	0.129 ± 0.038^a	0.084 ± 0.002^b	15.691 ± 0.683^a
CCE	0.395 ± 0.013^b	0.097 ± 0.003^b	14.829 ± 0.315^a
Ascorbic acid	0.004 ± 0.0002^a	0.002 ± 0.0004^a	-
Trolox®	0.016 ± 0.0012^a	0.009 ± 0.0006^a	-

Different letters in each column indicate significant differences at $p < 0.05$; CCA: *Cornukaempferia chayanii* aqueous extract; CCHE: 50% *Cornukaempferia chayanii* ethanolic extract; CCE: 95 % *Cornukaempferia chayanii* ethanol extract; n = 5.

Table 4: The α-glucosidase and α-amylase inhibitory activities of various extracts from *Cornukaempferia chayanii*

Sample	α-glucosidase IC_{50} (mg/dL)	α-amylase IC_{50} (mg/dL)
CCA	0.057 ± 0.002^a	0.025 ± 0.003^a
CCHE	0.216 ± 0.004^c	0.036 ± 0.001^a
CCE	0.183 ± 0.012^b	0.153 ± 0.025^b
Acarbose®	0.195 ± 0.018^b	0.173 ± 0.026^b

Different letters in each column indicate significant differences at $p < 0.05$; CCA: *Cornukaempferia chayanii* aqueous extract; CCHE: 50% *Cornukaempferia chayanii* ethanolic extract; CCE: 95 % *Cornukaempferia chayanii* ethanolic extract; n = 5.

The pancreas *α*-amylase and small intestine brush border α-glucosidase enzymes are mainly responsible for the action of carbohydrate hydrolyzing enzymes. This results in hyperglycemia or diabetes due to elevated blood glucose levels. Carbohydrate ingested is degraded into numerous monosaccharides before being absorbed into the blood vessel system due to the enzyme inhibitory activity.[18,19] Moreover, TPC and TFC have α-glucosidase and α-amylase inhibitory properties, which function as enzymes in the digestive system to improve and prevent various diseases.[20]

Conclusion

The findings of this study show that *Cornukaempferia chayanii* contains TPC and TFC. Furthermore, the medicinal plant possesses strong antioxidants, as well as *in vitro* α-glucosidase, and α-amylase inhibitory activities which could promote human health. Further research on the major active compound(s), including isolation, identification, purification, and *in vivo* studies, is required.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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