PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 10(3): 430–438
https://doi.org/10.14719/pst.2455

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RESEARCH ARTICLE

Antioxidant and antidiabetic properties of *Garcinia cowa* Roxb. extracts from leaves, fruit rind, and stem bark in different solvents

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OPEN ACCESS

ARTICLE HISTORY

Received: 21 February 2023
Accepted: 08 May 2023

Available online
Version 1.0 : 20 June 2023
Version 2.0 : 01 July 2023

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Wanna C, Boonman N, Phakpaknam S. Antioxidant and antidiabetic properties of Garcinia cowa Roxb. extracts from leaves, fruit rind, and stem bark in different solvents. Plant Science Today. 2023;10(3): 430–438. https://doi.org/10.14719/pst.2455

Abstract

The objective of this investigation was to assess the antioxidant and antidiabetic abilities of *Garcinia cowa* Roxb., a Thai native plant that has a history of use in traditional medicine in several countries. To achieve this goal, different plant parts, such as the leaves, fruit rind, and stem bark, were subjected to extraction with hexane, ethyl acetate, and 70% ethanol using the maceration method. The Folin-Ciocalteu technique was used to quantify the extracts total phenolic content (TPC). The antioxidant assays, including DPPH, ABTS and FRAP and antidiabetic properties through the α-amylase and α-glucosidase inhibitory capacities of different extracts from *G.cowa* were assessed. The results revealed that the stem bark extracted with ethyl acetate displayed the highest level of TPC at 153.68mg GAE/100g DW. Compared to the other extracts, the stem bark ethyl acetate extract demonstrated the highest antioxidant activity in DPPH, ABTS and FRAP assays with values of 436.86, 359.17 and 526.98µmol TE/100g DW, respectively. Further examination of the antidiabetic effects of stem bark extract in ethyl acetate exhibited the highest α-amylase and α-glucosidase inhibitory activities with IC_{50} values of 12.54 and 8.48mg/mL respectively. The findings of this research provide initial indications that *G. cowa* has both antioxidant and antidiabetic attributes and could be viewed as a potential therapeutic agent for managing diabetes.

Keywords

antioxidant; phenolic; α-amylase; α-glucosidase

Introduction

Diabetes mellitus (DM) is a progressive chronic metabolic disease characterized by chronically elevated blood glucose levels or hyperglycemia resulting from defects in insulin secretion, insulin action or both (1). Unhealthy food habits and modern lifestyles are the main contributing factors to the development of DM, which leads to excessive reactive oxygen species (ROS) production in the cells and causes oxidative stress. High ROS levels can damage biomolecules, leading to cellular dysfunction (2). Oxidative stress has been linked to various human diseases, such as diabetes, cardiovascular diseases, neurodegenerative and vascular diseases, cancer, aging, and obesity (3). Thailand has a high prevalence of diabetes, which is becoming a significant cause of death, posing a major public health problem (4). An effective therapeutic approach to manage diabetes is to reduce postprandial hypoglycemia (PPHG) by regulating and/or inhibiting carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase. The α-amylase breaks down long-chain carbohydrates, while α-glucosidase breaks down starch and disaccharides to glucose (5). Inhibition of α-glucosidase and

α-amylase can delay carbohydrate digestion. A prolonged glucose absorption rate that effectively lowers glucose (6, 7). Several drugs including acarbose, voglibose and miglitol have been used to control and treat diabetes by acting as α-glucosidase and α-amylase inhibitors. However, most antidiabetic drugs have adverse side effects (6, 8). Therefore, natural products are still essential as an alternative method for diabetes treatment.

Garcinia cowa Roxb., commonly known as Chamuang in Thailand, belongs to the Clusiaceae family (9). This edible plant is a rich source of oxygenated prenylated xanthones and benzophenones (10). Various biological and pharmacological activities have been observed in the chemical constituents in different parts of *G. cowa*. The stem bark has been found to possess antimalarial, antipyretic, antimicrobial and anti-inflammatory activities (11, 12). The leaves have been traditionally used for their anti-inflammatory, antitumor-promoting and antidiabetic properties (11, 13-15). The fruit rind has been reported to provide antiaflatoxigenic, antioxidant and α-glucosidase inhibitory activities (11, 16, 17). In addition, many xanthones are used in folk medicine for various purposes (18). The plant products are important sources of new chemical compounds leading to new drug discovery for more effective treatment and contain several active compounds against postprandial hypoglycemia (19). Plant polyphenols such as flavonoids, xanthones and tannic acids can inhibit the action of α-amylase and α-glucosidase enzymes, which reduces postprandial hyperglycemia (20-22).

However, little information has been reported regarding the α-amylase and α-glucosidase inhibitory activities of *G. cowa.* Therefore, this study aimed to investigate the antioxidant and antidiabetic properties, the total phenolic content and the α-amylase and α-glucosidase inhibitory activities of different *G. cowa* parts (leaf, fruit rind and stem bark) extracted with solvents of varying polarities (hexane, ethyl acetate and 70% ethanol). These findings could establish the potential effects of *G. cowa* for lowering postprandial glycemia and for disease prevention against free radical-mediated oxidative stress.

Materials and Methods

Chemical reagents

Sigma Chemical Co. (St. Louis, MO, USA) provided Folin Ciocalteu reagent, gallic acid, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) 2,2-diphenyl-1-picrylhydrazyl (DPPH) 6-hydroxy-2,5,7,8-tetramethyl chroman-2-Carboxylic acid (Trolox) 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), α-glucosidase from *Saccharomyces cerevisiae*, porcine pancreatic α-amylase, *p*-nitrophenyl-a-D-glucopyranoside (*p*-NPG) and acarbose. Analytical-grade chemicals and solvents were used for all other purposes.

Plant materials and extraction

The plant materials used in this study, including the leaves, fruits and stem bark of *G. cowa* (Fig. 1), was obtained from a local market in Nonthaburi province, in the central region of Thailand. The fruit rinds were separated from the rest of the plant material. Using taxonomic keys, a botanist at Bangkok Herbarium, Plant Varieties Protection, Department of Agriculture, Bangkok, Thailand, identified the plant species. The reference specimen had a voucher number coded as BK 065760.The plant material was cleaned with water, chopped into fragments, and then dried in a hot air oven for 48 hours at 40˚C. Extraction was carried out by using hexane, ethyl acetate, and 70% ethanol at room temperature (25˚C) for 24hours, following the methods published previously, with slight modifications (23, 24). The extracts were then filtered and a vacuum rotatory evaporator evaporated the solvents. The crude extracts were dissolved in dimethyl sulfoxide (5% DMSO) at a 100mg/ml concentration and stored at 4˚C for further analysis. The yields of the crude extracts are presented in Table 1 as % w/w of dry plant materials.

Determination of total phenolic content

The Folin-Ciocalteu method, with slight modifications, was used to evaluate the total phenolic content (TPC) of the extracts (25). In short, 20µL of extract solutions were combined with 100µL of 10% fresh Folin-Ciocalteu reagent in 96-well microplates and allowed to react for 5 min at room temperature (25˚C). Next, 80µL of 7.5% (w/v) sodium carbonate (Na_2CO_3) was added to the mixture, followed by incubation at room temperature (25˚C) for 30 min. The mixture's absorbance was measured at a wavelength of 765nm by using a microplate reader (SpectraMax M2). The experiment was conducted in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent per 100grams of dry weight (mg GAE/100g DW) by applying the gallic acid dilution series from 5-100mg/L for calibration.

Fig. 1. *Garcinia cowa* Roxb. (A) Whole plants; (B) Leaves; (C) Fruit rinds; (D) Stem barks.

Table 1. Percentage of extraction yields of *G. cowa* Roxb. with different solvents based on weight

Plant parts	Yields parentheses (% w/w)		
	Hexane	Ethyl acetate	70% Ethanol
Leaves	2.64	7.12	12.42
Fruit rind	0.91	3.23	8.54
Stem bark	1.56	5.27	10.63

Antioxidant activity

DPPH radical scavenging activity

The radical scavenging potential of the extracts was assessed by DPPH assay, following a published method (24) with some modifications. Briefly, a stock solution of 0.1mM DPPH was prepared by dissolving 0.4mg DPPH in 9.6mL of 80% ethanol. Then, 20µL of 30mg/mL sample extracts were combined with 180µL of 0.1mM DPPH solution in 96-well microplates. The mixture was briskly shaken and incubated for 30min at room temperature (25˚C) in the dark. After incubation, the reaction mixture was measured using a microplate reader (SpectraMax M2) at a wavelength of 515nm against a blank (ethanol). The percentages of DPPH radical scavenging activity inhibition of the samples were calculated according to the following equation:

% Inhibition of DPPH radical scavenging activity =

$[(A_{control} - A_{sample}) / A_{control}] \times 100$

Where $A_{control}$ = the absorbance of DPPH without samples

A_{sample} = the absorbance of DPPH with samples

The Trolox standard curve was used to determine values with concentrations ranging from 25-500µM, which were then expressed as micromoles of Trolox equivalent per 100g dry weight (µmol TE/100g DW). Data were presented as the averages of triplicate testing.

ABTS radical cation scavenging activity

The method described in a previous study (24) was used to assess the ABTS radical cation scavenging activity. To create a stable stock solution of ABTS radical cations, a 4mM aqueous solution of ABTS was reacted with 2.45mM potassium persulfate ($K_2S_2O_8$) at a ratio of 1:1 (v/v) in the dark at room temperature (25˚C) for 12-16 hours before use. The working solution was diluted with distilled water until an absorbance of 1.0 ± 0.02 was reached at 734nm. In a 96-well microplate, each 20µL of sample extract (30mg/mL) was mixed with 150µL of working solution. Immediately, the absorbance was measured at a wavelength of 734nm by using a microplate reader (SpectraMax M2) after 15min in the dark at room temperature (25˚C). The percentages of ABTS radical cation scavenging activity inhibition of the samples were calculated using the following equation:

% Inhibition of ABTS radical cation scavenging activity = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

Where $A_{control}$ = the absorbance of ABTS without samples

A_{sample} = the absorbance of ABTS with samples

The results were obtained by comparing with the Trolox calibration curve within a concentration range of 25-500µM and were reported as micromoles of Trolox equivalent per 100 g dry weight (µmol TE/100g DW). The experiments were performed in triplicate.

Ferric reducing antioxidant power assay

The ferric-reducing antioxidant power (FRAP) assay was modified based on a published method (24) that employs antioxidants as reductants in a colorimetric method linked to redox. The FRAP reagent was created by mixing 0.2M acetate buffer, pH 3.6 (0.2M acetic acid 46.3mL and 0.2M sodium acetate 3.7mL), 1mM 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) in 40mM HCl and 20mM $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1 and then incubated at 37˚C for 20min before use. The assay was carried out in 96-well microplates, and triplicate samples of 20µL of extracts (30mg/mL) were mixed with 150µL of FRAP reagent and incubated at room temperature (25˚C) for 10min. The decrease of ferric (Fe^{3+}) (which is colourless) to ferrous (Fe^{2+}) (which is blue) can be monitored by measuring the absorbance at 595nm using a microplate reader (SpectraMax M2) after 30min. The FRAP values were reported as micromoles of Trolox equivalent per 100grams dry weight (µmol TE/100g DW) using the Trolox dilution series ranging from 25-500µM for calibration.

In vitro antidiabetic activity

α-Amylase inhibitory activity

A modified method (26, 27) was used to evaluate the antidiabetic properties of *G. cowa* extracts by inhibiting α-amylase activity. In brief, 20µL of the extracts at different concentrations (3.75-60µg/mL) were mixed with 20µL of porcine pancreatic α-amylase 1U/mL and 80µL of 50mM sodium phosphate buffer (pH 6.8) in 96-well microplates. The mixture was incubated at 25˚C for 10min, followed by adding 50µL of 1% starch prepared in 50mM sodium phosphate buffer (pH 6.8). The reaction mixtures were incubated at 25˚C for 10min and then quenched by adding 30µL of dinitrosalicylic acid colour reagent. The mixtures were incubated in a boiled water bath until a yellowish-orange colour developed. After cooling to room temperature (25˚C), the mixtures were diluted to a 1:5 ratio with distilled water. The absorbance was measured using a microplate reader (SpectraMax M2) at a wavelength of 540nm. Acarbose was used as the positive control. The percentage inhibition of α-amylase activity for each extract was calculated using the following formula:

% Inhibition of α-amylase activity = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

Where $A_{control}$ = the absorbance of control (without samples)

A_{sample} = the absorbance of the samples

The results were expressed in terms of the concentration of the tested samples giving 50% inhibition of α-amylase activity (IC_{50}) estimated from the plots of the concentration v the inhibitory activity.

α-Glucosidase inhibitory activity

A slightly modified method (28) evaluated α-glucosidase inhibitory activity. In brief, 20µL of the solution containing different concentrations (3.75-60µg/mL) of each sample was mixed with 20µL of α-glucosidase enzyme from *Saccharomyces cerevisiae* (1U/mL) and 100µL of 50mM sodium phosphate buffer (pH6.8) in a 96-well microplate. The mixtures were incubated at 37˚C for 10min, followed by adding 50µL of 2mM *p*-nitrophenyl-a-D-glucopyranoside) (*p*-NPG), and the incubation continued for another 20min at 37˚C. The reaction was terminated by adding 50µL of 1mM sodium carbonate (Na_2CO_3)solution. Acarbose was used as a positive control. The absorbance

of the final solution was measured at a wavelength of 405nm using a microplate reader (SpectraMax M2). The α-glucosidase inhibition percentage was calculated using the following equation:

% Inhibition of α-glucosidase activity= $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where $A_{control}$ = the absorbance of control (without samples)

A_{sample} = the absorbance of the samples

The results were expressed in terms of the concentration of the tested samples giving 50% inhibition of α-glucosidase activity (IC_{50}) estimated from the plots of the concentration v the inhibitory activity.

Statistical analysis

All experiments had three iterations, and all analyses were performed in triplicate (n=3). The data were expressed as means ± standard deviations and analyzed by using the Statistical Package for Social Sciences (SPSS version 21). The mean values of the data were subjected to a one-way Analysis of Variance (ANOVA) and Duncan's new multiple tests determined the significance of the difference between means at 95% least significant difference ($p < 0.05$).

Results and Discussion

Extraction yields

The leaf, fruit rind, and stem bark of *G. cowa* extracted with different solvents of divergent polarity were prepared. The percentage yields of the crude extracts with hexane, ethyl acetate, and 70% ethanol are shown in Table 1. The weight -based percentage yields of the extracts obtained from the leaf, fruit rind, and stem bark were 2.64% to 12.42%, 0.91% to 8.54%, and 1.56% to 10.63% respectively. The results revealed that 70% ethanol extraction produced the highest amount of crude extract in all plant parts, followed by ethyl acetate, while the lowest yield was hexane. Leaf extract had the highest yield in 70% ethanol (12.42%), whereas fruit rind had the lowest yield from hexane extraction(0.91%).In addition, the percentage yields of this study were lower than those of previous investigations (29),which reported the yields of *G. cowa* leaf, fruit skin, and stem bark when extracted with ethanol to be 11.5%, 51.8% and 30.9%, respectively. The variation in extract yields was possibly dependent on the solvents used, the extraction techniques, the plant materials, and the differences in the chemical composition of the plant parts (30, 31).

Total phenolic content (TPC) and antioxidant activities

The Folin-Ciocalteu method was employed to determine the total phenolic content (TPC), and the antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays. Table 2 displays the results of total phenolic content and antioxidant activities for extracts obtained from the leaf, fruit rind and stem bark of *G. cowa* TPC and the antioxidant activities of each plant part extracted with ethyl acetate were higher than those extracted with 70% ethanol and hexane. The extracts for each plant part using different solvents showed statistical differences in the amount of TPC ranging from 42.87 to 153.68mg GAE/100g DW. Among the different plant parts, the highest TPC values were 63.55, 153.68 and 136.53mg GAE/100g DW found in hexane, ethyl acetate and 70% ethanol for stem bark, respectively. By contrast, the lowest TPC values were found in fruit rind for all solvents. These results agreed with previous results (29), which found that the TPC values of *G. cowa* leaf, fruit skin and stem bark were 17.172, 9.263, and 20.040mg/100g, respectively. Phenolic compounds are secondary metabolites that incorporate at least one phenol unit within their structure. These chemicals are naturally occurring in numerous plant species and serve as a defense mechanism by protecting plants from oxidative stress. They accomplish this by acting as a source of electrons or reducing agents, chelating metal ions, scavenging singlet oxygen, and deactivating lipid-free radical chains (32). Therefore, extracts containing high phenolic compound levels are required. The dissimilarity in solvent polarities accounted for the variations observed in the total phenolic content of each solvent. The degree of solvent polarity plays a critical role in enhancing the solubility of phenolic compounds. According to previous research, the isolation of more potent phenolic compounds was achieved with highly polar solvents (70% ethanol) and moderately polar solvents (ethyl acetate), whereas nonpolar solvents such as hexane produced less phenolic content than ethyl acetate and 70% ethanol (33). Furthermore, aqueous ethanol was more effective in extracting phenolic compounds than absolute ethanol (34).

The initial step in assessing the antioxidant potential of plant extracts is to use the DPPH free radical scavenging assay. This method gauges the plant extract's ability to donate an electron or hydrogen ion to DPPH to form a stable molecule(35).In this research, the scavenging activities of the extracts were highly variable, ranging from 124.75 to 436.86µmol TE/100g DW, as shown in Table 2. Across all solvents, the stem bark exhibited the highest DPPH radical scavenging activity with values of 175.55, 436.86 and 354.76µmol TE/100g DW for hexane, ethyl acetate and 70% ethanol, respectively, followed by the leaf.Conversely, the fruit rind demonstrated the lowest scavenging capacity of all solvents. Among the various solvents used, ethyl acetate extracts displayed a significantly higher DPPH radical scavenging activity.

Conversely, the lowest DPPH radical scavenging activities were exhibited by hexane. These results aligned with a previous study in which the *G.cowa* leaf in the ethyl acetate fraction had a higher percentage of inhibition against DPPH free radicals than the ethanol extract and n-hexane fraction (36). Similarly, in previous reports, the *G. madruno* (Kunth) leaf extracted with ethyl acetate provided the highest DPPH radical scavenging activity (37). Additionally, previous research revealed that the ethyl acetate extract from the stem bark of *G. fruticosa* Lauterb had a greater antioxidant potential than the n-hexane and methanol extracts (38). In contrast, the ethyl acetate extract of *G. mangostana* L. and the fruit rind exhibited the

highest antioxidant potential against DPPH(39).Hence, ethyl acetate extract is the most potent in terms of antioxidant activity as it has the highest DPPH radical scavenging activity.

Regarding the antioxidant potential of *G. cowa* extracts measured via ABTS assay, the findings agreed with the DPPH radical scavenging activity. It can be seen from the data in Table 2 that the ABTS cationic radical scavenging activity of *G. cowa* extracted with different solvents varied considerably among the plant parts, with a broad range from 135.64 to 359.17µmol TE/100g DW. The stem bark extracted with ethyl acetate exhibited 359.17µmol TE/100g DW and showed significantly higher scavenging ability than the other groups, followed by leaf, and fruit rind, respectively.70% ethanol and hexane showed similar activity in each plant part. This result was consistent with earlier researchers who found that the leaves of *G. madruno.* extracted with ethyl acetate had the highest ABTS radical scavenging activity, followed by methanol, hexane, and dichloromethane, respectively (37). The ABTS radical scavenging activity results aligned with previous research indicating that ethyl acetate was the optimal solvent for extracting bioactive compounds. Moreover, a previous study showed that α-mangostin had the highest concentration when extracted with ethyl acetate, followed by dichloromethane, ethanol, and water (40).Thus, solvent selection for extraction is based on chemical properties such as polarity and hydrophobicity to obtain the optimal bioactive compound yield (36).

Assessment of the antioxidant capacity of *G. cowa* by using FRAP assay relied on the reduction of the (Fe^{3+}-TPTZ) complex to a blue-coloured complex of (Fe^{2+}-TPTZ) (41). Likewise, the results of the FRAP assay exhibited the same trend as the DPPH and ABTS assay. As shown in Table 2, among the extracts of all plant parts, the higher reducing potential was offered in ethyl acetate and 70% ethanol rather than hexane. The reducing ability of stem bark extracted with ethyl acetate was significantly the highest at 526.98µmol TE/100g DW, followed by leaf, and fruit rind, respectively. Our results concurred with an earlier study, which showed that the reducing potential of ethanolic extract from the stem bark of *G. cowa* was highest at 26.837mmol Fe(II)/100g (29). Another similar study reported that leaf extract of *G. kydia.* Roxb. with ethyl acetate had the highest reducing power of EC_{50},equal to 12.389µg/mL (42). Moreover, these results corresponded to previous research carried out with *G. benthamiana., G. xanthochymus* and *G. hombroniana* in which the best antioxidant capacity was displayed by the ethyl acetate fraction (43,44). It is plausible that the high antioxidant activity observed in semi-polar and polar solvents, particularly in ethyl acetate, acetone, methanol and 80% (v/v) aqueous methanol, could be attributed to the presence of phenolics that are highly soluble in polar solvents (45).

The results indicate that the stem bark of *G.cowa.*is available source of TPC and displays superior antioxidant potential compared to the other plant parts examined. These findings are consistent with earlier reports that suggest a correlation between TPC values and the antioxidant activity of plant extracts (46). Secondary metabolites, such as phenolics, flavonoids, and tannins, are critical in determining the antioxidant activity of plant extracts (46). Based on the TPC and antioxidant activity results, ethyl acetate, 70% ethanol, and hexane are the most effective solvents for each plant part. Using different solvents in extracting from the same plant could lead to varying TPC and antioxidant activity values, which may be due to factors such as the type of compounds present, the polarity of the solvent used, and the plant part sampled (48). Therefore, the choice of extraction solvent significantly affects the yield and antioxidant activity of *G. cowa.* extracts. Additionally, the number and position of hydroxyl groups in the phenolic compounds could have influenced the antioxidant activity (49).

Table 2. Total phenolic content and antioxidant activities of *G. cowa* Roxb. leaves, fruit rind, and stem bark extracted with solvents of different polarity

Extracts	Total phenolic content (mg GAE/100 g DW)	Antioxidant activity (µmol TE/100 g DW)		
		DPPH assay	ABTS assay	FRAP assay
Hexane				
Leaves	56.40 ± 0.71^{b}	138.20 ± 1.69^{b}	147.35 ± 4.36^{b}	185.47 ± 2.83^{b}
Fruit rind	42.87 ± 0.62^{a}	124.75 ± 1.49^{a}	135.64 ± 3.61^{a}	162.96 ± 2.62^{a}
Stem bark	63.55 ± 0.66^{c}	175.55 ± 1.18^{d}	164.94 ± 2.42^{d}	218.31 ± 1.84^{c}
Ethyl acetate				
Leaves	125.22 ± 1.57^{g}	364.85 ± 2.96^{h}	285.43 ± 2.15^{h}	444.85 ± 2.97^{g}
Fruit rind	103.61 ± 0.88^{e}	296.63 ± 1.16^{f}	176.38 ± 1.98^{e}	368.74 ± 1.23^{f}
Stem bark	153.68 ± 1.12^{i}	436.86 ± 2.60^{i}	359.17 ± 2.40^{i}	526.98 ± 1.54^{h}
70% Ethanol				
Leaves	117.33 ± 0.65^{f}	276.53 ± 1.24^{e}	205.08 ± 1.60^{f}	332.28 ± 2.30^{e}
Fruit rind	87.44 ± 1.56^{d}	154.54 ± 1.62^{c}	157.50 ± 0.77^{c}	276.50 ± 1.99^{d}
Stem bark	136.53 ± 1.08^{h}	354.76 ± 3.71^{g}	276.51 ± 1.51^{g}	443.89 ± 1.71^{g}

All data were expressed as mean ± standard deviation. Different letters in the column indicate significant differences at $p < 0.05$ using one-way ANOVA followed by Duncan's new multiple range test (DMRT)
DW = dry weight; GAE = gallic acid equivalent; TE = trolox equivalent

α-Amylase and α-glucosidase inhibitory activities

The ability of extracts from *G. cowa* leaf, fruit rind and stem bark in different solvents to inhibit α-amylase and α-glucosidase activities in the context of their antidiabetic potential was tested, with acarbose used as the positive control. The results were presented as IC_{50} values, with lower values indicating stronger inhibition. This study is the first report of inhibitory activity against α-amylase and α-glucosidase for *G. cowa* crude extracts. The α-amylase inhibitory activity of all extracts is shown in Table 3.All tested extracts inhibited α-amylase at levels ranging from 12.54 to 42.31µg/mL. The stem bark extract obtained with ethyl acetate exhibited significantly higher α-amylase inhibitory activity than the other extracts, with an IC_{50} value of 12.54µg/mL. The current study found that a high amount of TPC was extracted with ethyl acetate, and the extracts had potent antidiabetic properties. The effectiveness of TPC in inhibiting α-amylase activity depends on the number and position of the hydroxyl groups (50).

The range of anti-α-glucosidase enzymatic activity in all extracts was 8.48 to 38.41µg/mL, as described in Table 3. Among the different plant parts, the ethylacetate for stem bark extract exhibited the strongest significant results at inhibiting α-glucosidase with IC_{50} of 8.48µg/mL, followed by leaf and fruit rind, respectively. Also, the 70% ethanol and hexane showed the same trends in each plant part. This study was similar to previous studies that reported that the ethyl acetate extract of *G. cowa* leaf had IC_{50} with a value of 21.40µg/mL(15).However, the inhibitory effects of extracts on α-amylase and α-glucosidase showed lower activity than acarbose as the positive control, with IC_{50} values of 6.57 and 2.68µg/mL.

Moreover, the hexane extracts showed weak inhibition on both enzymes, consistent with previous reports on the stem extracts of *Lasia spinosa* L. in n-hexane, which produced IC_{50} values larger than 400µg/mL (51). Possibly due to the ethyl acetate extract containing more active compounds that can inhibit α-amylase and α-glucosidase activities. Therefore, our findings had different IC_{50} values depending on the plant parts used and the solvents employed for extraction.

Correlation between TPC, antioxidant and antidiabetic potentials

The Pearson correlation coefficients between the TPC, antioxidant and antidiabetic activities of *G. cowa* extract from the leaf, fruit rind and stem bark in different solvents are depicted in Table 4. As shown in this study, there was a considerable positive correlation. The stem bark extracted with ethyl acetate had a high TPC content, correlated with better anti-α-amylase and anti-α-glucosidase activities. Several studies have confirmed a positive correlation between the antioxidants present, such as polyphenols, and α-amylase and α-glucosidase inhibition (5,26,28,38). The results suggested that these extracts could prevent the hydrolysis of carbohydrates into monosaccharides and absorbance through the intestine, leading to low postprandial glucose levels in the blood (5,22). An earlier investigation of phytochemical content in parts of *G. cowa* extracts reported the presence of xanthones, phloroglucinols, benzophenones, depsidones, flavonoids, tannin, terpenoids, steroids and saponins (10,14). Xanthones are the principal markers in the *Garcinia* genus. The five xanthones found in the stem bark included 7-O-methylgarcinone, cowanin, cowanol, cowaxanthone and β-mangostin; these may be responsible for inhibiting α-amylase and α-glucosidase as hydrogen bond donors and acceptors (14,15,17). These results were related to the chemical compounds in the extract that could attenuate the oxidative radicals and inhibit both enzymes synergistically (38). Other research studies show that flavonoids have antioxidant activity and can demonstrate the inhibition of α-amylase and α-glucosidase activity due to the substitution of hydroxyl groups, thereby increasing the number of free phenolic groups, which will increase the ability to inhibit both enzymes (22). Moreover, tannins can inhibit α-glucosidase and antioxidant activities because they consist of hydroxyl groups and can bind to proteins to make complexes (21,26).

Table 3. IC_{50} for α-amylase and α-glucosidase inhibitory effects of *G. cowa* Roxb. leaves, fruit rind, and stem bark extracted with different solvents

Extracts	IC_{50} (µg/mL)	
	α-Amylase	α-Glucosidase
Hexane		
Leaves	34.89 ± 0.61^{g}	31.36 ± 0.38^{h}
Fruit rind	42.31 ± 0.83^{h}	38.41 ± 0.45^{i}
Stem bark	28.72 ± 0.46^{e}	23.54 ± 0.53^{e}
Ethyl acetate		
Leaves	25.15 ± 0.26^{d}	19.34 ± 0.68^{d}
Fruit rind	32.75 ± 0.55^{f}	26.21 ± 0.30^{f}
Stem bark	12.54 ± 0.49^{b}	8.48 ± 0.27^{b}
70% Ethanol		
Leaves	28.22 ± 0.39^{e}	24.24 ± 0.44^{e}
Fruit rind	34.56 ± 0.27^{g}	29.66 ± 0.35^{g}
Stem bark	18.05 ± 0.64^{c}	14.43 ± 0.16^{c}
Acarbose	6.57 ± 0.29^{a}	2.68 ± 0.15^{a}

All data were expressed as mean ± standard deviation. Different letters in the column indicate significant differences at $p < 0.05$ using one-way ANOVA followed by Duncan's new multiple range test (DMRT)

Conclusion

Based on the findings of this study, it is concluded that various parts of *G. cowa* such as leaf, fruit rind and stem bark in different solvents, have potential total phenolic content as well as antioxidant and antidiabetic properties. The results showed that the stem bark extract, using ethyl acetate demonstrated the highest total phenolic content, DPPH and ABTS radical scavenging activities, reducing power ability and inhibitory effects against α-amylase and α-glucosidase. According to the data, phenolic compounds may mainly contribute to antioxidant and antidiabetic activities. However, more research is needed to understand the bioactive components and their biological properties in context of *G. cowa.*

Table 4. The correlation coefficient of total phenolic content (TPC), antioxidant and antidiabetic activities of *G.cowa* Roxb. leaves, fruit rind, and stem bark in different solvents

Assays	TPC	DPPH	ABTS	FRAP	α-Amylase	α-Glucosidase
TPC	1					
DPPH	0.953	1				
ABTS	0.903	0.940	1			
FRAP	0.978	0.980	0.933	1		
α-Amylase	0.881	0.880	0.929	0.874	1	
α-Glucosidase	0.895	0.904	0.928	0.900	0.994	1

Acknowledgements

The authors gratefully acknowledge the Institute for Research and Development for the full funding of this research project and providing all the necessary support for this work through the Faculty of Science and Technology, Suan Sunandha Rajabhat University, Bangkok, Thailand.

Authors contributions

CW conducted the design of the study, carried out the experiments and drafted the manuscript. NB performed the statistical analysis and revised the manuscript. SP participated in the plant collection and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors here with declare no conflict of interest.

Ethical issues: None.

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