Vasorelaxing effect of *Garcinia cowa* leaf extract in rat thoracic aorta and its underlying mechanisms

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ABSTRACT

Background and aim: The leaves of *Garcinia cowa* (*G. cowa*) are used in Thai traditional medicine to improve blood circulation. However, there is no scientific evidence to confirm this therapeutic claim. Here, we investigated the vasorelaxing effect and its underlying mechanisms of an aqueous extract of *G. cowa* leaves in rat thoracic aortic rings.
Materials and methods: Dried leaves of *G. cowa* were extracted with water, followed by phytochemical analysis. Vascular reactivity experiments were performed in isolated rat thoracic aortic rings using an organ bath system. The results were recorded using the data acquisition system Power Lab.
Results: Phytochemical analysis showed that the leaves of *G. cowa* are rich in polyphenols and flavonoids, especially kaempferol, vitexin, and isovitexin. The *G. cowa* leaf extract caused a concentration-dependent relaxation of aortic rings. This effect was attenuated by denudation of the endothelium, or by pre-treatment of the aortic rings with L-NAME, ODQ, indomethacin, or glibenclamide, but not with TEA.
Conclusion: This study indicates that *G. cowa* leaf extract induces vasorelaxation through both endothelium-dependent and endothelium-independent manners. Its mechanism of action mainly involves the production of nitric oxide and prostanoids, as well as opening ATP-sensitive K^+ channels. The vasorelaxing effect of *G. cowa* leaf extract is probable promoted by the action of flavonoids.

1. Introduction

Hypertension or high blood pressure is a condition in which the blood vessels have persistently raised pressure, with systolic and diastolic blood pressure levels exceeding 140 and 90 mmHg, respectively. Untreated hypertension significantly increases the risk factor for developing cardiovascular diseases (CVDs), which are the leading cause of death globally, accounting for 31% of all annual global mortality.[1] Despite the several types of antihypertensive drugs currently available, successful treatment for hypertension remains difficult to achieve, and long-term administration of some antihypertensive drugs can produce side effects like muscle cramp, dizziness, headache, abnormal heart rate, etc.[2] Accordingly, over the past decades, researchers have shifted their attention towards plant-based medications because they have multi-target efficacy, have fewer side effects, are rich in resources, and are low in cost.

Garcinia cowa (*G. cowa*) Roxb, locally known as Chamuang in Thai, is an endemic traditional plant widely distributed in the southern part of Thailand and throughout Malaysia and Indonesia.[3] The leaves of *G. cowa* are popularly used for cooking and have long been used in traditional folk medicine for the treatment of indigestion, for improving blood circulation, and as an expectorant.[4] Previous *in vitro* studies showed that the ethanolic extract of *G. cowa* leaves has antioxidant, antihyperlipidemic, and anti-Alzheimer's disease activities.[5–7] Furthermore, the methanolic extract of *G. cowa* leaves was found to be effective against gastrointestinal pathogenic bacteria and to produce cytotoxic effects against cancer

cells.[8,9] Phytochemical analysis of an aqueous extract of *G. cowa* leaves previously revealed the presence of hydroxycitric acid and its lactone, polyphenolic glycoside, and flavonoids.[10,11] Polyphenols and flavonoids have long been reported as antihypertensive agents. These compounds induce vasorelaxation via various pathways, such as by acting on the NO/cGMP pathway, activating the PGI$_2$/cAMP pathway, activating K$^+$ channels, and lowering intracellular Ca^{2+} concentrations.[12,13] Based on this information, the present study aimed to investigate the vasorelaxing effect of an aqueous extract of *G. cowa* leaves and to explore its mechanism of action on thoracic aortic rings.

2. Materials and methods

2.1. Plant material and extraction

Fresh leaves of *G. cowa* Roxb. were collected in Songkhla province, Thailand. The plant material was identified by Nirun Vipunngeun. A voucher specimen (Collecting no. RSU. PHAR.08) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Pathum Thani, Thailand. The fresh leaves were carefully washed in tap water to remove any dust and oven-dried at 45 °C for 48 h. The dried leaves were ground into a coarse powder using a blender. The powdered dry leaves were added to distilled water at a ratio of 1:10 (w/v) and heated at 70 °C for 24 h. The solution was filtered through muslin cloth and cotton followed by lyophilization to obtain a reddish-brown powder (a yield of about 8.49% of *G. cowa* fresh leaves). The extraction powder was collected in an amber-glass bottle and kept at −4 °C until further use.

2.2. Phytochemical constituent analysis

2.2.1. Total phenolic content analysis

The total phenolic content of the *G. cowa* leaf extract was determined via the Folin−Ciocalteu method as previously described.[14] Briefly, 5 mg/mL of *G. cowa* leaf extract was dissolved in absolute ethanol and diluted to 100 μg/mL with distilled water. One hundred microliters of diluted *G. cowa* leaf extract was mixed with 500 μL of Folin−Ciocalteu's phenol reagent and 400 μL of aqueous Na$_2$CO$_3$ (75 g/L) in a test tube. The mixtures were incubated in a dark room for 30 min at room temperature. The absorbance was measured at 765 nm using a spectrophotometer. The test was analyzed in triplicate. A standard curve of gallic acid was prepared in ethanol in the range of 4−80 μg/mL (R$_2$ = 0.999). The total phenolic content is expressed as milligrams of gallic acid equivalent weight (GAE) per gram of dry weight mass.

2.2.2. Total flavonoid content analysis

The total flavonoid content of the *G. cowa* leaf extract was determined via an aluminum chloride (AlCl$_3$) colorimetric method as previously described.[14] Briefly, 100 mg/mL of *G. cowa* leaf extract was dissolved in 80% ethanol, followed by dilution to 1 mg/mL with distilled water. Five hundred microliters of diluted *G. cowa* leaf extract was mixed with 1500 μL of 95% ethanol, 100 μL of 10% AlCl$_3$, 100 μL of 1 M potassium acetate, and 2800 μL of distilled water in a test tube. The mixture was vortexed and allowed to incubate in a dark room for 30 min at room temperature. The absorbance was measured at 415 nm using a spectrophotometer. The test was analyzed in triplicate. A standard curve of quercetin was prepared in 80% ethanol in the range 20−100 μg/mL (R$_2$ = 0.999). The total flavonoid content is expressed as milligrams of quercetin equivalent weight (QE) per gram of dry weight mass.

2.2.3. Liquid chromatography mass spectrometry (LC-MS) analysis

The chemical constituent profile of the *G. cowa* leaf extract was determined via LC-MS using an Agilent HPLC (6200 series) coupled with a G6545A MS-Q-TOF LC-MS mass analyzer. The analysis was performed on a column compartment (G7116B model) set at 25 °C, with a gradient of 0.1% formic acid in HPLC-grade water and acetonitrile, at a flow rate of 0.2 mL/min. The mass traces were detected by a diode array detector (G7117A) set at wavelengths 210, 254, 266, and 330 nm. MS/MS analysis was operated in the negatively ionized electrospray (ESI) mode with nitrogen as the drying gas at a pressure of 13 psi. The mass spectra were obtained over the *m/z* range of 100−1000 mAU. The constituents were identified by comparing their mass spectra with an online database and the TOF LC-MS data processer.

2.3. In vitro cytotoxicity study

The cytotoxic effect of the *G. cowa* leaf extract on Vero cells was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with doxorubicin as the positive control, as previously described.[15] Briefly, the Vero cells were harvested and seeded onto 96-well plates at a density of 2 × 10^4 cells/well. After allowing the cells to adhere for 24 h, *G. cowa* leaf extracts in a concentration range of 62.5−1000 μg/mL were dispensed, followed by incubation for 72 h. After incubation, the viability of the cells was determined by adding 100 μL of 0.5 mg/mL MTT, followed by incubation at 37 °C for 30 min in darkness. The absorbance was measured at 570 and 650 nm using a microplate reader spectrophotometer. Cytotoxicity is expressed as the IC$_{50}$ value.

2.4. Animals

Male Wistar rats weighing 200−300 g were supplied by Nomura Siam International Co., Ltd. The animals were acclimatized for a week at the Southern Laboratory Animal Facility, Faculty of Science, Prince of Songkla University and housed under a 12 h dark and 12 h light cycle at a temperature of 22 ± 3 °C with a relative humidity of 60−70%. All the animals were allowed to access standard food and water *ad libitum.* The animal experiment was performed in accordance with the ethical principles and guidelines for the care and use of laboratory animals set by the National Research Council of Thailand. The study protocol was approved by Animal Care and Use Committee of Prince of Songkla University (Approval project code: 2563-01-058 dated 22.02.2020).

2.5. Thoracic aortic ring preparation

Rats were anesthetized with Anesthal (60 mg/kg, i. p. injection) and euthanized by cervical dislocation. The thoracic aorta was immediately isolated and placed in a 37 °C oxygenated Krebs Henseleit solution (composition in mM: NaCl 118.3, KCl 4.7, CaCl$_2$ 1.9, MgSO$_4$·7H$_2$O 0.45, KH$_2$PO$_4$ 1.18, NaHCO$_3$ 25.0, glucose 11.66, Na$_2$EDTA 0.024, and ascorbic acid 0.09), followed by careful removal of the adhesive connective tissue and fat tissue. The isolated thoracic aorta was then cut into ring segments of approximately 3−4 mm in length, and endothelium-denuded aortic rings were prepared by gently rubbing the inner surface with fine stainless steel. The aortic rings were then mounted horizontally between two parallel stainless-steel hooks, suspended in a 20 mL organ bath filled with Krebs Henseleit solution, maintained at 37 °C, and continuously bubbled with a carbogen gas (95% O$_2$−5% CO$_2$). One hook was fixed at the bottom of the organ bath, and the other was connected to a force displacement transducer to measure the isometric force. Isometric contraction and relaxation were

recorded and analyzed using the PowerLab Data Acquisition System with LabChart 8 AD instruments. The aortic rings were equilibrated for 60 min with a basal tension of 1 g, and the bath solution was changed every 15 min. After equilibration, the aortic rings' integrity was assessed by pre-contraction with phenylephrine (3 μM), followed by the addition of acetylcholine (30 μM) to induce relaxation. In the endothelium-intact aortic rings, the acetylcholine had to induce more than 80% relaxation, whereas endothelium-denuded aortic rings had to show no relaxation. Lastly, the aortic rings were washed several times until the tension returned to basal tension, and they were allowed to equilibrate for 45 min before starting the experimental protocols.

2.6. Experimental protocols

To determine the vasorelaxing effect of *G. cowa* leaf extract, the endothelium-intact and endothelium-denuded aortic rings under basal tension of 1 g were contracted with phenylephrine. When the contractile response reached a plateau, the cumulative concentration—response curves (0.01—3.0 mg/mL) of *G. cowa* leaf extract were determined.

In order to determine the mechanism involved in *G. cowa* leaf extract-induced vasorelaxation, the endothelium-intact aortic rings were incubated for 30 min with 10 μM *Nω*-Nitro-ʟ-arginine methyl ester (a non-selective nitric oxide synthase inhibitor), 10 μM 1*H*-[1,2,4] oxadiazolo [4,3-α] quinoxaline-1-one (a soluble guanylate cyclase inhibitor), 10 μM indomethacin (a non-selective cyclooxygenase inhibitor), 10 μM glibenclamide (an ATP-sensitive potassium channel blocker), or 1 mM tetraethylammonium (a non-selective Ca^{2+}-activated potassium channel blocker). After incubation, the aortic rings were precontracted with phenylephrine, followed by the addition of a cumulative concentration of *G. cowa* leaf extract.

2.7. Drugs

Acetylcholine chloride (ACh), *Nω*-Nitro-ʟ-arginine methyl ester (ʟ-NAME), phenylephrine (PE), tetraethylammonium (TEA), glibenclamide (Gliben), 1*H*-[1,2,4] oxadiazolo [4,3-α] quinoxaline-1-one (ODQ), and indomethacin (Indo) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All drug solutions were dissolved in a solution containing NaCl 9 g/L, NaH_2PO_4 0.19 g/L, and ascorbic acid 0.03 g/L.

2.8. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Relaxation responses to *G. cowa* leaf extract are presented as a percentage decrease of phenylephrine contraction. The maximal relaxation response (R_{max}) and the negative logarithm of the *G. cowa* leaf extract concentration that produced 50% of the maximal relaxation response (pED_{50}) were generated using the GraphPad Prism Software Version 9.0 (San Diego, CA, USA). The statistical differences between two groups were evaluated by Student's *t*-test, whereas one-way ANOVA followed by a Tukey's *post hoc* test was employed to determine the statistical differences among multiple groups. All the results were analyzed using the GraphPad Prism Software Version 9. A *P* value of <0.05 was considered to indicate statistically significant difference.

3. Results

3.1. Phytochemical constituents of G. cowa leaf extract

The total phenolic content and total flavonoid content of the

G. cowa leaf extract were measured via the Folin—Ciocalteu method and $AlCl_3$ colorimetric method, respectively. The total phenolic content of *G. cowa* leaf extract was 103.56 ± 2.08 mg GAE/g, and the total flavonoid content was 15.04 ± 0.32 mg QE/g. Furthermore, the phytochemical constituent profile was identified by LC-MS analysis. The LC-MS chromatogram of the *G. cowa* leaf extract is shown in Fig. 1. The molecular formula and content of the compounds (peak area%) are presented in Table 1. The LC-MS analysis revealed that the *G. cowa* leaf extract contained several flavonoids, including kaempferol, vitexin, isovitexin, catechin, isomengiferin, and eriodictyol. The kaempferol, vitexin, and isovitexin were the major flavonoids of the *G. cowa* leaf extract.

3.2. Cytotoxic effect of G. cowa leaf extract

The *G. cowa* leaf extract concentration ranging from 62.5 to 1000 μg/mL did not produce a toxic effect on Vero cells, whereas doxorubicin as the positive control showed a marked cytotoxic effect.

3.3. Vasorelaxing effect of G. cowa leaf extract

The vasorelaxing effect of *G. cowa* leaf extract was determined in isolated thoracic aortic rings obtained from four animals. The *G. cowa* leaf extract induced a concentration-dependent relaxation of both endothelium-intact and endothelium-denuded aortic rings precontracted with phenylephrine. However, removal of the endothelium caused a significant shift in the concentration—response curve (C—R curve) of the *G. cowa* leaf extract to the right and a decrease in the vasorelaxation response from a concentration of 0.03 mg/mL to 1.0 mg/mL, as well as a significant decrease in the maximal relaxation response (Fig. 2A and B). The pED_{50} values of the *G. cowa* leaf extract obtained in endothelium-intact and endothelium-denuded aortic rings were 3.35 ± 0.16 and 2.91 ± 0.03, respectively, whereas the R_{max} values were 88.08 ± 5.46% and 54.86 ± 8.04%, respectively (Table 2).

3.4. The effect of different blockers on the vasorelaxing action of G. cowa leaf extract

To investigate the involvement of the NO/cGMP pathway in the *G. cowa* leaf extract-induced vasorelaxation, the endothelium-intact aortic rings were incubated with eNOS inhibitor ʟ-NAME or ʟ-NAME and sGC inhibitor ODQ. Either incubation, with ʟ-NAME or with ʟ-NAME and ODQ, caused a significant shift in the C—R curve of *G. cowa* leaf extract to the right, as well as a significant decrease in the maximal relaxation response (Fig. 3A). The pED_{50} value of the *G. cowa* leaf extract obtained in endothelium-intact aortic rings was reduced from 3.44 ± 0.14 to 3.07 ± 0.10 in the presence of ʟ-NAME and to 3.04 ± 0.05 in the presence of ʟ-NAME and ODQ. To the same extent, R_{max} was also reduced from 87.76 ± 5.47% to 72.69 ± 6.19% in the presence of ʟ-NAME and to 69.76 ± 3.87% in the presence of ʟ-NAME and ODQ (Table 2).

In order to determine the role of prostanoids in the *G. cowa* leaf extract-induced vasorelaxation, the endothelium-intact aortic rings were incubated with the COX inhibitor indomethacin. In the presence of indomethacin, the vasorelaxing effect of *G. cowa* leaf extract was significantly decreased, and the R_{max} was reduced from 92.60 ± 5.46% to 58.35 ± 4.16% (Fig. 3B and Table 2).

In addition, the participation of potassium channels was elucidated by incubating the endothelium-intact aortic rings with K_{ATP} channel blocker glibenclamide or K_{Ca} channel blocker TEA. Glibenclamide incubation caused a significant decrease in the vasorelaxing effect of *G. cowa* leaf extract at a high concentration, which reduced the R_{max} from 85.83 ± 4.11% to 57.61 ± 2.91% (Fig. 4A and Table 2), while TEA incubation had no effect (Fig. 4B and Table 2).
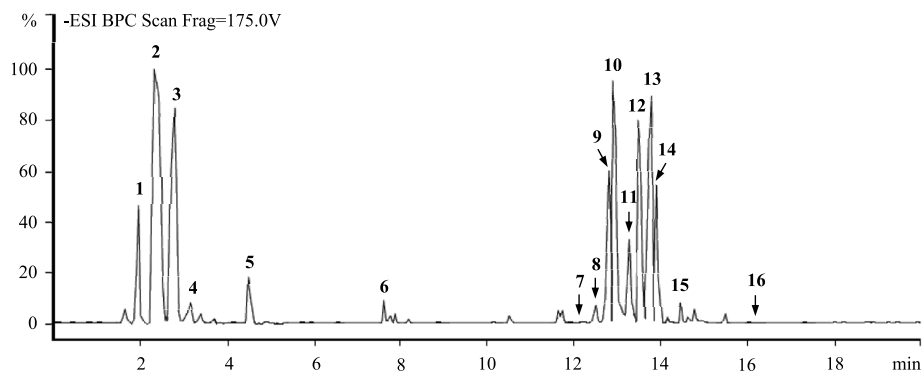
Fig. 1. LC-MS chromatogram of *G. cowa* leaf extract.

Table 1
Phytochemical constituents in the *G. cowa* leaf extract identified by LC-MS.

| No.	Name of Compounds	RT (min)	Molecular Formula	Fragment ions (m/z)	Score (DB>)	Diff (DB, ppm)	Area (%)
1	Hydroxycitric acid	1.95	$C_6H_8O_8$	207.02	96.39	−3.71	4.91
2	2-Methyl-5-(methylthio) thiophene	2.34	$C_6H_8O_8$	189.01	81.17	−3.57	21.55
3	Hydroxycitric acid	2.76	$C_6H_8O_8$	207.02	95.35	−4.61	13.36
4	Melibiitol	3.13	$C_{12}H_{24}O_{11}$	343.12	95.83	−0.20	1.12
5	Citric acid	4.49	$C_6H_8O_7$	191.02	99.27	−1.37	2.07
6	Phenyl glucoronide	7.62	$C_{12}H_{14}O_7$	315.07	97.89	−0.96	0.63
7	(±)-Catechin	12.19	$C_{15}H_{14}O_6$	289.07	99.77	−0.58	0.10
8	Vitexin 4′-O-glucoside-2″-O-rhamnoside	12.49	$C_{33}H_{40}O_{19}$	739.21	98.02	0.40	0.62
9	Kaempferol 4′-glucoside	12.81	$C_{21}H_{20}O_{11}$	447.09	98.69	−0.98	5.24
10	Vitexin 4′-O-galactoside	12.93	$C_{27}H_{30}O_{15}$	593.15	98.50	−2.79	13.71
11	Kaempferol 4′-glucoside	13.28	$C_{21}H_{20}O_{11}$	447.09	98.79	−0.70	3.93
12	Kaempferol 3-rhamnoside-(1->2)-rhamnoside	13.50	$C_{27}H_{30}O_{14}$	577.16	97.14	−1.72	11.05
13	Kaempferol 3-rhamnoside-(1->2)-rhamnoside	13.77	$C_{27}H_{30}O_{14}$	577.16	98.79	−1.72	11.66
14	Isovitexin	13.90	$C_{21}H_{20}O_{10}$	431.10	98.20	−1.51	5.09
15	Isomengiferin	14.46	$C_{19}H_{18}O_{11}$	421.08	98.40	−0.29	0.66
16	Eriodictyol	16.20	$C_{15}H_{12}O_6$	287.06	97.81	−0.72	0.08

Noted: RT = retention time; DB = database.
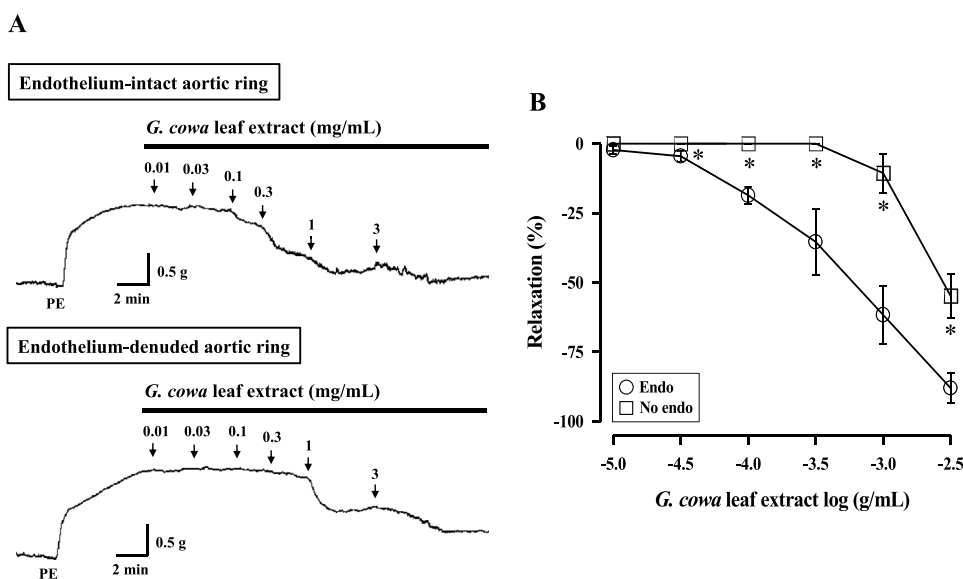
Fig. 2. Original isometric force recordings showing the PE-precontracted endothelium-intact and endothelium-denuded aortic rings treated with cumulative concentration of *G. cowa* leaf extract (**A**). The cumulative concentration−response curves of *G. cowa* leaf extract-induced vasorelaxation on PE-precontracted endothelium-intact (Endo) and endothelium-denuded (No endo) aortic rings (**B**). The cumulative concentration−response curve of *G. cowa* leaf extract in endothelium-intact aortic rings was assigned as a control. Values represent the mean ± SEM of 4 animals. Significant at *$P < 0.05$ as compared to the endothelium-intact aortic ring group.

Table 2
The pED$_{50}$ values and R$_{max}$ values for *G. cowa* leaf extract-induced vasorelaxation with different antagonists.

| Aortic ring condition/antagonist	pED$_{50}$	R$_{max}$ (%)
First line screening		
Endothelium-intact aortic ring	3.35 ± 0.16	88.08 ± 5.46
Endothelium-denuded aortic ring	2.91 ± 0.03^a	54.86 ± 8.04^a
NO-cGMP cascade mechanisms		
Control	3.44 ± 0.14	87.76 ± 5.47
L-NAME	3.07 ± 0.10^a	72.69 ± 6.19^a
L-NAME + ODQ	3.04 ± 0.05^a	69.76 ± 3.87^a
Prostaglandin cascade mechanisms		
Control	3.53 ± 0.16	92.60 ± 5.46
Indomethacin	3.49 ± 0.10	58.35 ± 4.16^b
ATP-sensitive K$^+$ channel		
Control	3.31 ± 0.15	85.83 ± 4.11
Glibenclamide	3.02 ± 0.14	57.61 ± 2.91^b
Ca^{2+}-activated K$^+$ channel		
Control	3.41 ± 0.18	91.47 ± 4.71
TEA	3.21 ± 0.15	89.85 ± 4.07

Noted: pED$_{50}$ = the negative logarithm of the half of effective dose; R$_{max}$ = maximal relaxation; results are expressed as mean $\pm$ SEM (n = 4); ^{a} and ^{b} indicate significant level at $P < 0.05$ and 0.01, respective compare to their corresponding control group.

4. Discussion

This study demonstrated that *G. cowa* leaf aqueous extract is composed of several polyphenols and flavonoids such as kaempferol, vitexin, isovitexin, catechin, isomengiferin, and eriodictyol. Kaempferol, vitexin, and isovitexin are the major flavonoids contained in the *G. cowa* leaf extract. These flavonoids, particularly kaempferol, were found to have a potent vasorelaxant effect.[16] The results of this study clearly reveal that *G. cowa* leaf extract relaxes the vessel in both endothelium-dependent and endothelium-independent manners. The mechanism of the relaxation response is mediated by the production of nitric oxide and prostanoids, as well as the opening ATP-sensitive K$^+$ channels.

The vascular endothelium plays an important role in regulating vascular tone. It releases three main vasoactive substances to dilate blood vessels: nitric oxide (NO), prostanoids, and EDHF.[17–19] There has been much evidence to indicate that the bioactive components derived from plants, especially flavonoids such as quercetin, kaempferol, and resveratrol, can induce vasorelaxation though the promotion of the production and release of these vasodilators from the endothelium.[12,20,21] The results show that *G. cowa* leaf extract-induced vasorelaxation is endothelium-dependent because the effect was significantly decreased when the endothelium was removed. However, it is interesting that in the absence of endothelium, high concentrations of *G. cowa* leaf extract continued to show a vasorelaxing effect, suggesting that the *G. cowa* leaf extract has a direct effect on vascular smooth muscle; the mechanism of this relaxation will be further investigated.
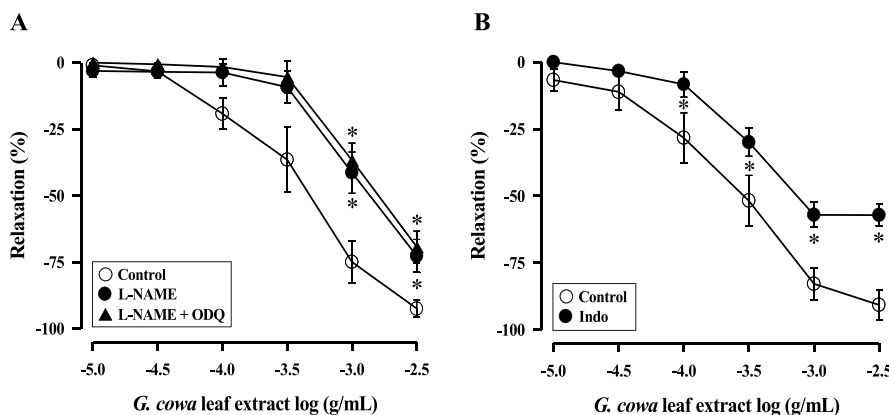
Fig. 3. The cumulative concentration−response curves of *G. cowa* leaf extract-induced vasorelaxation in the presence of **(A)** L-NAME and L-NAME plus ODQ or **(B)** indomethacin (Indo) on PE-precontracted endothelium-intact aortic rings. Values represent the mean $\pm$ SEM of 4 animals. Significant at $*P < 0.05$ as compared to the control group.
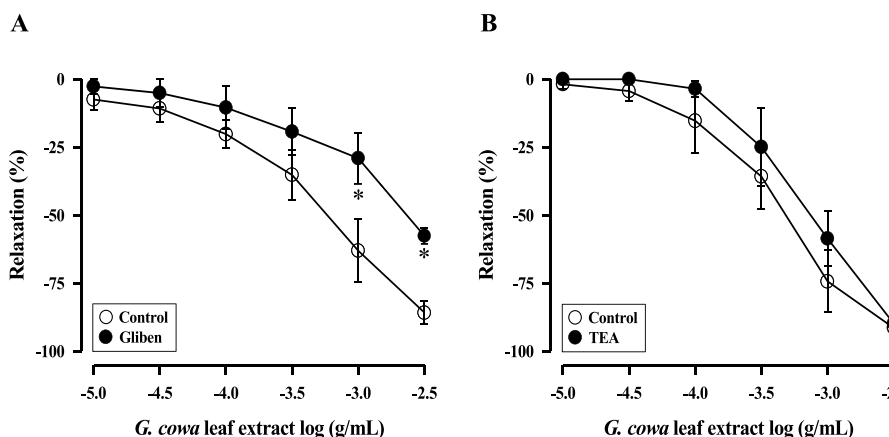
Fig. 4. The cumulative concentration−response curves of *G. cowa* leaf extract-induced vasorelaxation in the presence of **(A)** glibenclamide (Gliben) or **(B)** tetraethylammonium (TEA) on PE-precontracted endothelium-intact aortic rings. Values represent the mean $\pm$ SEM of 4 animals. Significant at $*P < 0.05$ as compared to the control group.

NO is the most potent endothelium-derived relaxing factor; it is produced from L-arginine and oxygen by a catalytic enzyme, endothelial nitric oxide synthase (eNOS), and subsequently diffuses into the vascular smooth muscle cell where it stimulates soluble guanylate cyclase (sGC) to produce cyclic GMP, which, in turn, induces the relaxation of vascular smooth muscle.[22,23] This study shows that inhibiting eNOS activity by L-NAME, as well as inhibiting sGC activity by ODQ, caused a significant decrease in G. cowa leaf extract-induced vasorelaxation, indicating that the vasorelaxing effect of G. cowa leaf extract is partially mediated through the release of NO from the endothelium.

In addition to NO, a prostanoid, PGI$_2$, is an important vasodilator derived from the endothelium, synthesized from arachidonic acid by enzymatic reaction of the cyclooxygenase (COX) pathway. PGI$_2$ induces vascular smooth muscle relaxation by stimulating the adenylate cyclase to produce cyclic AMP that leads to a decrease in intracellular Ca^{2+}, which, in turn, induces relaxation of vascular smooth muscle.[24,25] The present finding shows that the vasorelaxing effect of G. cowa leaf extract was reduced when the thoracic aortic rings were pre-incubated with COX inhibitor indomethacin, suggesting that G. cowa leaf extract can stimulate prostanoid production and subsequently induce vasorelaxation.

Moreover, the vascular tone is also regulated by EDHF. This factor induces hyperpolarization at the vascular smooth muscle, which, in turn, induces relaxation. EDHF is associated with the opening of K$^+$ channels, including Ca^{2+}-activated K$^+$ channels (K$_{Ca}$) and ATP-sensitive K$^+$ channels (K$_{ATP}$).[26,27] The results of this study reveal that the K$_{ATP}$ inhibitor glibenclamide decreased the vasorelaxing effect of G. cowa leaf extract, but the K$_{Ca}$ inhibitor TEA did not. These findings indicate that G. cowa leaf extract induces vasorelaxation by opening K$_{ATP}$.

Several studies have demonstrated that flavonoid-rich extracts cause vasorelaxation.[28–31] This study proved that G. cowa leaf extract contains several flavonoids, especially kaempferol, vitexin, and isovitexin. Various evidence has suggested that kaempferol is a potent vasorelaxant, and its effects are associated with both endothelium-dependent and endothelium-independent pathways. Kaempferol relaxes vessels via the activation of NO–cGMP–PKG signaling, potentiation of bradykinin-induced relaxation, and activation of large-conductance Ca^{2+}-activated potassium channels (BK$_{Ca}$).[32–34] More recently, vitexin and isovitexin were reported to possess antihypertensive effects. Vitexin relaxed phorbol ester-induced vascular contraction primarily through inhibiting MEK activity and the subsequent ERK1/2 phosphorylation.[35] Meanwhile, the vasorelaxing effect of isovitexin is mediated by the stimulation of NO release, leading to the activation of small-conductance Ca^{2+}-activated potassium channels (SK$_{Ca}$), and the upregulation of Kir 6.1 ATP-sensitive K$^+$ channels.[36] According to the results, it is possible that the vasorelaxing effect of G. cowa leaf extract results from the synergistic action of flavonoids contained in the extract, especially kaempferol, vitexin, and isovitexin.

In addition to the vasorelaxing effect, the cytotoxic effect of G. cowa leaf extract was evaluated. The result revealed that G. cowa leaf extract did not produce toxic effects against Vero cells, suggesting that it is safe to use. However, further toxicological studies are needed to investigate this.

5. Conclusion

The present study provides evidence confirming that G. cowa leaf extract has vasorelaxant properties. The mechanism of action mainly involves the production of nitric oxide and prostanoids, as well as opening K$_{ATP}$. Furthermore, the phytochemical analysis revealed that the leaves of G. cowa are rich in polyphenols and flavonoids, especially kaempferol, vitexin, and isovitexin. These

compounds are probably responsible for the vasorelaxing effect of the G. cowa leaf extract. However, the vasoactive compounds should be further identified, and additional animal models are required to validate the in vivo antihypertensive effect of G. cowa leaf extract.

Declaration of competing interest

There are no conflicts of interest.

Acknowledgement

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