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Original Article

In Vitro Antidiabetic and *In Vivo* Hypoglycaemic Activities and Toxicity of *Canarium zeylanicum* Bark Extracts

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Abstract: Aqueous bark extract of *Canarium zeylanicum* (Retz.) Blume (Burseraceae) is an antidiabetic remedy in indigenous medicine. Its antidiabetic potential has not been scientifically evaluated. In this study, antidiabetic activity and toxicity of the bark of *C. zeylanicum* were evaluated using a selected *in vitro* and *in vivo* set of experiments. A hot water extract (HWE) and an organic solvent extract (OSE) of the bark and respective negative and positive controls were subjected to *in vitro* α-amylase- and α-glucosidase-inhibitory and brine shrimp lethality assays. *In vivo* hypoglycaemic activity and hepato- and nephro-toxicities were evaluated by twice daily administration of each extract and the controls to 8 groups (n=10) of normoglycaemic Wistar rats for 14 days. Data of *in vivo* studies were statistically analysed using Minitab 16 with *P*- <0.05. IC_{50} values of HWE, OSE and the positive control in the α-amylase-inhibitory assay were 65.3 ± 18.5, 8.4 ± 4.1 and 0.04 ± 0.1 µg/ml, respectively; corresponding IC_{50} values in the α-glucosidase-inhibitory assay were 2.9 ± 0.1, 21.6 ± 0.5 and 0.01 ± 0.0 µg/ml. Brine shrimp lethality assay revealed that the extracts were non-toxic. *In vivo* studies revealed that extracts possess hypoglycaemic activity with higher percentage reduction (38.2 ± 12.9) of fasting blood sugar by OSE, high glucose tolerance, and the extracts were neither hepatotoxic nor nephrotoxic. Lupeol is one of the counterparts for the antidiabetic actions while some active compounds could not be resolved further. Results indicate that *C. zeylanicum* is a potential source for developing effective non-toxic antidiabetic drugs.

Keywords: *Canarium zeylanicum* antidiabetic plants, glucose tolerance

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by increased blood glucose levels due to inefficient insulin secretion or activity [1]. Uncontrolled blood glucose levels for a long time lead to diabetic complications such as peripheral neuropathy, nephropathy, loss of vision, etc. It was estimated that globally, there were 463 million diabetics in 20-79 age group in 2019 and this number is increasing at an alarming rate [2]. International Diabetes Federation has projected that the number of patients would be 700 million by the year 2045 [2]. The total global expenditure estimated for treating diabetes in 2019 was USD

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760 billion while the projection for the year 2045 is USD 845 billion [3]. Increasing the number of unhealthy and huge expenses on health will badly affect the global economic development. To treat diabetes, a number of medicines belonging to several classes of glucose lowering agents are available in allopathic medicine. Biguanides, sulphonylureas, meglitinides, thiazolidinediones (TZDs), alpha-glucosidase inhibitors and DPP-4 inhibitors are widely used allopathic oral hypoglycaemic agents. Each drug class has its own cellular mechanism/s for glucose lowering. Some of the antidiabetic drug classes have one or more different physiological actions to bring down plasma glucose levels. However, their prolonged use is associated with adverse effects of which some are serious [4]. The associated side effects of the current antidiabetic drugs used in allopathic medicine limit their usage. On the other side, traditional medical practices are gaining popularity globally due to their accessibility, affordability, safety and efficacy. However, traditional medicinal practices vary widely according to culture, understanding of people and accessibility to the medicines [5].

Some people in Asian and African countries largely depend on traditional remedies for controlling diabetes and such remedies are primarily based on plants [6,7]. Search for plant-based antidiabetic compounds is attractive as they may have alternative and safe effects on diabetes. *Canarium zeylanicum* (Retz.) Blume of the family Burseraceae is a deciduous large tree. Much branched *C. zeylanicum* trees may grow up 30 m height [8]. Traditionally different parts of *C. zeylanicum* are used in medicinal and non-medicinal applications [9]. In indigenous medicine, *C. zeylanicum* is used in both external and internal medicinal preparations [9]. The bark of the tree is used as an astringent and antiseptic [9,10]. Decoction prepared using the bark is used as a gargling agent in bleeding and spongy gums. Chronic ulcers and fistula are dressed with an ointment prepared involving bark extracts [10]. Bark is used as an aromatic, stomachic, astringent, febrifuge and antiperiodic [10]. It is used in infective fevers and malaria [10]. The resinous gum is used medicinally as a gargle for pyorrhoea and halitosis [10] while non-medicinally it is used in fumigation and lightening [8,10]. Aqueous extract of *C. zeylanicum* stem bark is used in indigenous system of medicine for treating diabetes [9-11], where 120 ml of extract is given twice daily for diabetics [11]. However, its antidiabetic potential has not been scientifically evaluated before. The aim of the study was *in vitro* and *in vivo* evaluation of the potential of the use of bark extracts of *C. zeylanicum* as an antidiabetic remedy.

Materials and methods

Collection of plant material

Bark of *Canarium zeylanicum* was collected from Kegalle, Sabaragamuwa Province, Sri Lanka. The botanical identity of the plant was confirmed by a Field Biologist, Sri Lanka and a voucher specimen of the same (FAHS/CZ/2013/01) was deposited at the Department of Pharmacy, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka.

Chemicals, other materials and instruments

The chemicals required in *in vitro* assays were of analytical grade and purchased from Sigma-Aldrich Chemie Gmbh (Germany) and Merck (Germany). Brine shrimp eggs were obtained from a commercial fish stall, Kandy, Central Province, Sri Lanka. In *in vitro* assays, absorbance was measured on Multiskan GO microplate spectrometer, Thermo Scientific, USA. For *in vivo* studies, reagent kits for liquid glucose oxidase, liquid AST (SGOT), liquid ALT (SGPT) and creatinine were purchased from Pointe Scientific INC, USA. Blood parameters were measured on Pointe 180 semi-automated chemistry analyzer, USA.

Experimental animals

The experimental protocol was approved by the Ethics Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka (Ethics Review Committee reference number: EC-12-062). Healthy 2-3 months old male Wistar rats weighing 200-250 g [12] were purchased from Medical Research Institute, Colombo, Sri Lanka. All the animals were housed and

treated in accordance with the internationally accepted laboratory animal use and care [13] and the Guidelines for Ethics Review of Research Proposals Involving Animals in Sri Lanka [14]. The animals were kept in polypropylene cages with wire mesh lids at 22 ± 2ºC with a 12 h light/ dark cycle, with free access to water and a World Health Organization (WHO) recommended pelleted diet. Before the experiments, the rats were allowed to acclimatize in the laboratory environment for 10 days.

Extraction of plant material

Bark of *C. zeylanicum* was sliced into small (1-1.5 cm x 2.5-3.5 cm) pieces, shade-dried for 4 weeks inside a room and powdered using a mechanical home-kitchen grinder to obtain a coarse powder. Powdered bark (50 g) was boiled in deionized water (1.4 l) for 4 h until the final volume reaches 175 ml with time-to-time water replacement for 2 h and then controlling the burner temperature. The extract (175 ml) was filtered and the filtrate was freeze-dried to obtain the hot water extract (HWE). Powdered plant material (50 g) was extracted with methanol and dichloromethane (1:1) mixture (1.5 l) at room temperature for 24 h x 2. The filtrate was rotary evaporated at 40°C to dryness thus the organic solvent extract (OSE) was obtained.

***In vitro* bioassays**

α-Amylase-inhibitory assay

The α-Amylase-inhibitory assay was performed on both HWE and OSE as described by Nickavar *et al*. [15] with modifications to the concentrations of the plant extracts (250-3000 µg/ml) dissolved in 1% (v/v) dimethyl sulfoxide (DMSO). Acarbose and 1% (v/v) DMSO were used as the positive and the negative control, respectively. The inhibition percentages (equation 1) of α-amylase corresponding to different concentrations of extracts were assessed and the concentration of the extract (µg/ml) necessary to decrease the absorbance of α-amylase by 50% (IC_{50}) was determined [16].

Inhibition percentage = [{Absorbance (negative control) - Absorbance (test)} / Absorbance (negative control)] x 100 (1)

α-Glucosidase-inhibitory assay

The α-Glucosidase-inhibitory assay was performed on both HWE and OSE as described by Sharp *et al*. [17]. Acarbose and 1% (v/v) DMSO were used as the positive and the negative control, respectively. The inhibition percentages (Equation 1) of α-glucosidase corresponding to different concentrations of plant extracts (62.5-1000 µg/ml) in 1% (v/v) DMSO were assessed and the concentration of the extract (in µg/ml) necessary to decrease the absorbance of α-glucosidase by 50% (IC_{50}) was determined [16].

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was performed on both HWE and OSE as described by Meyer *et al*. [18]. Brine shrimp eggs were allowed to hatch in continuously aerated artificial seawater illuminated with light using a 20-W bulb. After 24 h, hatched nauplii (10) were drawn with artificial seawater (1 ml) and transferred to a cell of a 24-well plate. To each cell containing nauplii, respective plant extract (concentration, 50-2500 µg/ml) in 1% (v/v) DMSO in artificial seawater (1 ml) was added and kept under the same light source. After 24 h, the number of surviving nauplii was counted. In this assay, 4-hydroxy-2-methylquinoline and 1% (v/v) DMSO in artificial seawater were considered as the positive and the negative control, respectively. The lethality percentages (equation 2) of brine shrimps corresponding to different concentrations of extracts were assessed and the concentration of the extract (µg/ml) necessary to exhibit 50% lethality (LC_{50}) was determined.

Lethality percentage = (number of dead nauplii / 10) x 100 (2)

***In vivo* bioassays**

***Acute oral toxicity study* [19] *and dose selection* [20]**

Five concentrations (200, 400, 800, 1600 and 3200 mg/kg) of both extracts, HWE and OSE were administered to 10 groups (n=6) of normoglycaemic rats. There were only 30 animals and tested for one extract first and then with a 14-day interval they were reused for the next extract. Each dose of HWE and OSE was mixed with 2 ml of distilled water separately, and orally

administered to 12-h fasted rats. Rats were closely observed at 2 and 4 h after the administration of test material for any adverse signs (signs of toxicity, changes in appearance and physical activities). Blood sugar levels of rats who did not show any adverse signs were measured before and after 2 and 4 h of administration of extracts. The lowest, intermediate and the highest doses of each extract were determined based on the extract showing about 20 % reduction in the sugar level in the single-dose administration.

Multiple-dose administration study [20, 21]

Eighty normoglycaemic rats were randomly divided into 8 groups (n=10). The identified lowest, intermediate and highest doses of HWE and OSE were mixed with 2 ml of distilled water separately and administered twice daily orally to rats assigned randomly to different groups and on a normal diet for 2 weeks. Tolbutamide 22.5 mg/kg body weight/dose [10] and Distilled water were used as the positive and the negative control, respectively. Fasting blood sugar (FBS), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum creatinine level were measured in the 12-h fasted animals before administering the test materials and then after 7 days and on the 15th day. The weight of each animal was measured on the day before starting the test and on the day of blood sample collection. Rats were observed for any adverse signs during and after 2 days of the test.

Oral glucose tolerance test

On the 15th day of multiple dose administration of test materials, oral glucose tolerance test (OGTT) [12, 21] was performed. In the OGTT, blood sugar levels of rats were measured at 1, 2 and 4 h after an oral glucose load of 2.5 g/kg body weight [12,22].

Phytochemical studies

Based on the results, OSE of *C. zeylanicum* was selected for carrying out phytochemical studies.

Phytochemical screening

OSE was screened for the presence of alkaloids (Iodine and Dragendorff tests), flavonoids (Pew's, Shinoda, Sodium hydroxide, Sulphuric acid and Lead acetate tests), glycosides (Keller-Kiliani test), phenols (Ferric chloride test), saponins (Foam test) and sterols (Liebermann-Burchard, Salkowski and Sulphuric acid tests) [23].

Bioassay guided fractionation

OSE was subjected to α-amylase and α-glucosidase inhibitory assay-guided fractionation: sonication based solvent fractionation (Separated to methanol and dichloromethane fractions), the highest active methanol fraction then subjected to *n*-butanol and water solvent-solvent partitioning, the highest active water fraction then subjected to a fractionation step using semi-preparative HPLC (column C18, particle size 5 µm, diameter 21.2 mm, length 250 mm, mobile phase ACN+H_2O, 10 ml/min). Each semipreparative HPLC fraction was observed for separation of compounds using analytical HPLC profiles (C18, Sunfire, particle size 5 µm, diameter 4.6 mm, length 250 mm, mobile phase ACN+H_2O, 1 ml/min).

Isolation and identification of chemical compounds responsible for antidiabetic activities

OSE (12 g) of *C. zeylanicum* was shaken with *n*-hexane. Based on the results of α-amylase and α-glucosidase inhibitory assays, hexane non-soluble fraction was subjected to 2 successive fractionation steps under gravity using silica gel (70-230 and 230-400 mesh sizes) employing gradient elution (first by hexane-ethylacetate-methanol solvent and then by Hexane-dichloromethane-methanol solvents) followed by fractionation using sephadex LH-20 (MeOH). Structures of the compounds were elucidated using FTIR, ^{1}H and ^{13}C NMR, DEPT, COSY, HMBC, HMQC, NOESY and GCMS experiments.

Statistical analysis

All experimental results are presented as mean ± SD. In *in vivo* experiments, significant differences between groups were determined by ANOVA using Minitab (version 17). The difference between experimental groups

was analysed using Dunnett's test. *P* value of less than 0.05 was considered to be significant.

Results and discussion

Through hot water and organic solvent extraction procedures, the yields resulted were 10.12% and 7.85%, respectively.

α-Amylase-and α-glucosidase enzyme-inhibitory assays

α-Amylase and α-glucosidase are enzymes that are involved in the digestion of starch and glycogen resulting in glucose. Inhibition of such enzymes will slow down the glucose generation after a carbohydrate rich diet leading to reduction in glucose uptake, and this is considered a strategy for the control of diabetes [24]. In this study, the inhibitory activity of *C. zeylanicum* extracts on α-amylase and α-glucosidase enzymes was examined. Both HWE and OSE showed high α-amylase (Figure 1) and α-glucosidase (Figure 2) inhibitory

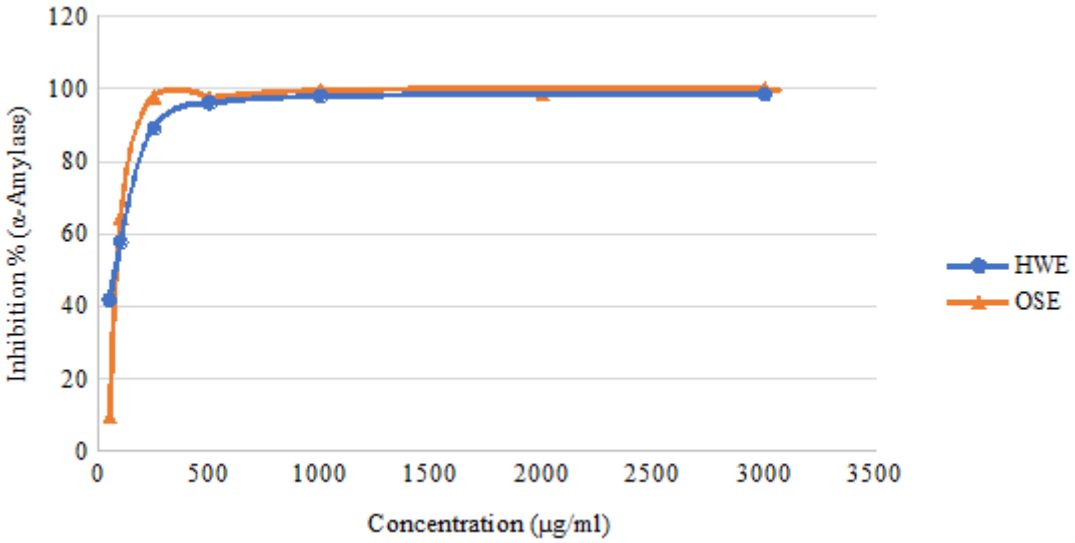


Figure 1. α-Amylase-inhibitory activities of *C. zeylanicum* extracts (HWE, hot water extract; OSE, organic solvent extract)

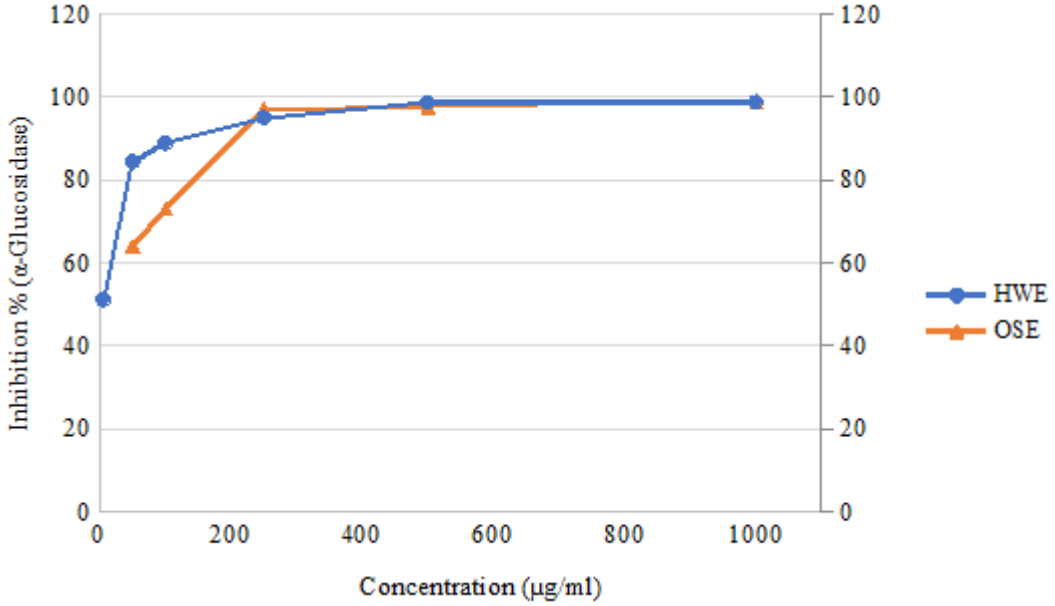


Figure 2. α-Glucosidase-inhibitory activities of *C. zeylanicum* extracts (HWE, hot water extract; OSE, organic solvent extract)

activity in concentration dependent manner. The IC_{50} values of both extracts and the positive control for α-amylase and α-glucosidase enzyme are shown in Table 1.

Brine shrimp lethality bioassay

The cytotoxicity of hot water extract (HWE) and organic solvent extract (OSE) of *C. zeylanicum* was evaluated using brine shrimp lethality bioassay and LC_{50} values were 1750 and 750 µg/ml, respectively, for each extract. Thus, each extract of *C. zeylanicum* was non-toxic because a plant extract is considered cytotoxic if its IC_{50} value is < 100 mg/ml in the brine shrimp lethality assay [25].

Acute oral toxicity study and dose selection

Acute oral toxicity study showed that the administered doses (200-3000 mg/kg doses) exerted no observable signs of toxicity on rats. Animals were neither dead nor had any external signs of adverse effects and were physically active after the administration of *C. zeylanicum* extracts. However, it must be stated that the internal organs of the animals were not examined for signs of toxicity. The reduction of fasting blood sugar (FBS) levels in normoglycaemic rats upon single-dose administration of extracts for dose selection is shown in Table 2. The reduction of FBS levels was dose dependent. However, at high doses (1600 and 3200 mg/kg) of OSE, hypoglycaemic activity did not appear to be dose dependent.

The results of dose selection study showed > 20 % reduction in FBS levels indicating the potential hypoglycaemic activity [20] of *C. zeylanicum* extracts. Based on the observation of 20% or more reduction in the FBS level, the doses of extracts, 200, 400 and 800 mg/kg body weight, were selected as the lowest, intermediate and the highest doses for multiple doses administration of both HWE and OSE for 14 days.

Table 1. IC_{50} values (µg/ml) of *C. zeylanicum* extracts and respective positive controls on α-amylase- and α-glucosidase-inhibitory assays

Assay	IC_{50} (µg/ml)		
	HWE	OSE	Control
α-Amylase inhibitory assay	65.3 ± 18.5	8.4 ± 4.1	0.04 ± 0.1[a]
α-Glucosidase inhibitory assay	2.9 ± 0.1	21.6 ± 0.5	0.01 ± 0.0[a]

HWE, hot water extract; OSE, organic solvent extract; [a], acarbose

Table 2. Reduction of fasting blood sugar (FBS) levels in normoglycaemic rats upon single-dose administration of *C. zeylanicum* extracts for dose selection

Dose (mg/kg)	FBS reduction %			
	HWE		OSE	
	after 2 h	after 4 h	after 2 h	after 4 h
200	1.7 ± 5.9	20.0 ± 10.3	12.8 ± 7.7	20.3 ± 9.1
400	4.7 ± 7.4	16.7 ± 3.9	11.2 ± 7.4	21.2 ± 6.9
800	6.8 ± 6.2	19.6 ± 10.3	28.7 ± 10.4	25.2 ± 13.7
1600	14.2 ± 8.9	23.2 ± 6.7	1.80 ± 5.2	1.20 ± 11.9
3200	9.2 ± 9.5	25.8 ± 9.0	10.2 ± 8.1	8.9 ± 3.2

HWE, hot water extract; OSE, organic solvent extract; Values are the mean ± standard deviation of six (n = 6) determinations

Multiple-dose administration study

There was no significant difference between the body weights of rats in different groups after administration of test materials (extracts, positive and negative controls) for two weeks. It indicated that the feeding pattern of rats in each test group was not influenced by the administration of test material. Further, there was neither observable changes in the external appearance of animals nor abnormal behaviours during and after 2 days of the test. Percentage reduction in the test parameters (FBS, SGOT, SGPT and serum creatinine) after administration of extracts to each group for 7 days is summarized in Table 3. When compared with the negative control (distilled water administered) group, the rats treated with certain concentrations of *C. zeylanicum* extracts showed significant reduction in FBS levels. The rats receiving OSE showed a higher reduction in FBS levels (21.5 ± 11.1 % and 25.8 ± 17.2 % at 200 and 800 mg/kg doses, respectively) than the rats receiving HWE (10.7 ± 28.0 % and 17.5 ± 13.6 % at 200 and 400 mg/kg doses, respectively). However, the group receiving the standard drug tolbutamide did not show a reduction in FBS levels after 7 days.

Percentage reduction in FBS, SGOT, SGPT and serum creatinine after administration of extracts to each group for 14 days is summarized in Table 4.

Both HWE and OSE of *C. zeylanicum* at their highest dose (800 mg/kg) showed significant reductions in FBS levels of rats compared to reduction in FBS levels of rats which were administered distilled water. The highest reduction in FBS levels (38.2 ± 12.9 %) after 14 days was observed in the OSE administered group and it was higher than that in the tolbutamide administered group (35.4 ± 15.6 %).

After 7 and 14 days of administration of *C. zeylanicum* extracts to rats, there was no significant difference in SGPT, SGOT and serum creatinine levels between control and treatment groups. This indicates that there was neither liver nor renal damage on rats administered with *C. zeylanicum* extracts for 14 days.

Oral glucose tolerance test (OGTT)

The results of the OGTT are shown in Table 5. All the tested doses of HWE and OSE of *C. zeylanicum*, except the 800 mg/kg dose of OSE, showed lesser percentage increase of blood sugar levels in respective rat groups after 1 h of glucose load, compared with positive and negative controls. This observation indicates that the rise in sugar level was controlled by

Table 3. Percentage reduction in FBS, SGOT, SGPT and serum creatinine values after 7 days administration of *C. zeylanicum* extracts, the positive and the negative controls to rats

Group	% Reduction			
	FBS	S. Cr.	SGOT	SGPT
Distilled water	5.90 ± 20.1	8.0 ± 10.3	6.4 ± 20.7	15.4 ± 9.4
200 mg/kg HWE	10.7 ± 28.0	6.3 ± 12.8	1.5 ± 15.3	16.7 ± 18.8
400 mg/kg HWE	17.5 ± 13.6*	3.3 ± 7.0	4.5 ± 9.20	15.4 ± 21.4
800 mg/kg HWE	16.0 ± 12.5	4.7 ± 14.7	1.6 ± 12.8	16.0 ± 12.0
200 mg/kg OSE	21.5 ± 11.1*	1.3 ± 10.2	0.5 ± 17.7	16.9 ± 15.4
400 mg/kg OSE	15.3 ± 21.8	1.7 ± 5.7	7.4 ± 17.9	17.2 ± 29.3
800 mg/kg OSE	25.8 ± 17.2*	1.8 ± 5.6	-2.5 ± 14.4	14.7 ± 16.5
22.5 mg/kg Tolbutamide	0.60 ± 21.3	3.0 ± 11.3	1.7 ± 12.8	19.2 ± 38.0

FBS, fasting blood glucose; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; S. Cr., serum creatinine; HWE, hot water extract; OSE, organic solvent extract; *, values significantly different from the negative control group; Values are the mean ± standard deviation of ten (n = 10) determinations, $P < 0.05$

Table 4. Percentage reduction in FBS, S. Cr., SGOT and SGPT values after 14 days administration of *C. zeylanicum* extracts, the positive and the negative controls to rats

Group	% Reduction			
	FBS	S. Cr.	SGOT	SGPT
Distilled water	1.80 ± 20.5	2.3 ± 10.7	10.7 ± 26.8	11.2 ± 04.7
200 mg/kg HWE	16.0 ± 45.3	10.7 ± 14.5	9.7 ± 12.2	10.7 ± 22.1
400 mg/kg HWE	21.5 ± 18.5	5.0 ± 08.0	6.2 ± 10.5	11.0 ± 18.3
800 mg/kg HWE	28.6 ± 18.5*	1.0 ± 15.0	16.0 ± 17.8	11.8 ± 16.0
200 mg/kg OSE	7.10 ± 11.5	1.3 ± 10.2	9.2 ± 22.1	6.6 ± 20.7
400 mg/kg OSE	12.1 ± 9.2	5.0 ± 08.1	4.6 ± 15.5	11.5 ± 21.2
800 mg/kg OSE	38.2 ± 12.9 *	1.0 ± 13.4	5.2 ± 19.8	10.9 ± 28.7
22.5 mg/kg Tolbutamide	35.4 ± 15.6	2.7 ± 14.3	14.3 ± 10.5	15.6 ± 09.3

FBS, fasting blood glucose; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; S. Cr., serum creatinine; HWE, hot water extract; OSE, organic solvent extract; *, values significantly different from the control group; Values are the mean ± standard deviation of ten (n = 10) determinations, $P < 0.05$

Table 5. Results of OGTT *C. zeylanicum* extracts, the positive and the negative controls to rats

Group	Basal glucose level	blood sugar level after 1 h of glucose load	% increase of blood sugar level after 1 h due to glucose load	blood sugar level after 2 h	blood sugar level after 4 h
Distilled water	92.2 ± 12.5	166.0 ± 17.1	83.8 ± 35.7	124.9 ± 10.9	110.0 ± 21.4
200 mg/kg HWE	93.9 ± 20.1	145.2 ± 15.1	58.4 ± 23.2	118.1 ± 13.8	107.6 ± 16.9
400 mg/kg HWE	101.4 ± 28.8	154.1 ± 11.1	64.8 ± 53.3	136.7 ± 24.9	134.0 ± 16.7
800 mg/kg HWE	79.0 ± 25.9	126.5 ± 19.7	64.8 ± 42.4	115.4 ± 19.9	115.4 ± 21.6
200 mg/kg OSE	104.9 ± 9.20	145.2 ± 19.6	39.4 ± 23.5	128.6 ± 15.9	110.5 ± 10.3
400 mg/kg OSE	109.4 ± 12.3	147.7 ± 11.1	36.4 ± 16.6	124.1 ± 11.2	112.6 ± 10.5
800 mg/kg OSE	75.1 ± 18.9	145.7 ± 17.0	105.1 ± 52.6	131.5 ± 25.1	119.9 ± 23.2
22.5 mg/kg Tolbutamide	81.9 ± 19.6	142.1 ± 37.5	75.0 ± 35.6	94.60 ± 30.2	77.90 ± 28.2

HWE, hot water extract; OSE, organic solvent extract; Values are the mean ± standard deviation of ten (n = 10) determinations, $P < 0.05$

Table 6. Phytochemical screening of the organic solvent extract (OSE) of *C. zeylanicum*

Chemical constituent	Test	Presence/ Absence
Alkaloids	Iodine test	-
	Dragendorff test	-
Flavonoids	Pew's test	-
	Shinoda test	√
	Sodium hydroxide test	-
	Sulphuric acid test	-
	Lead acetate test	√
Glycosides	Keller-Kiliani test	-
Phenols	Ferric chloride test	√
Saponins	Foam test	-
Sterols	Leiebermann-Burchard test	√
	Salkowski test	√
	Sulphuric acid test	-
Tannins	Test for tannins	√

√, presence; _, absence-

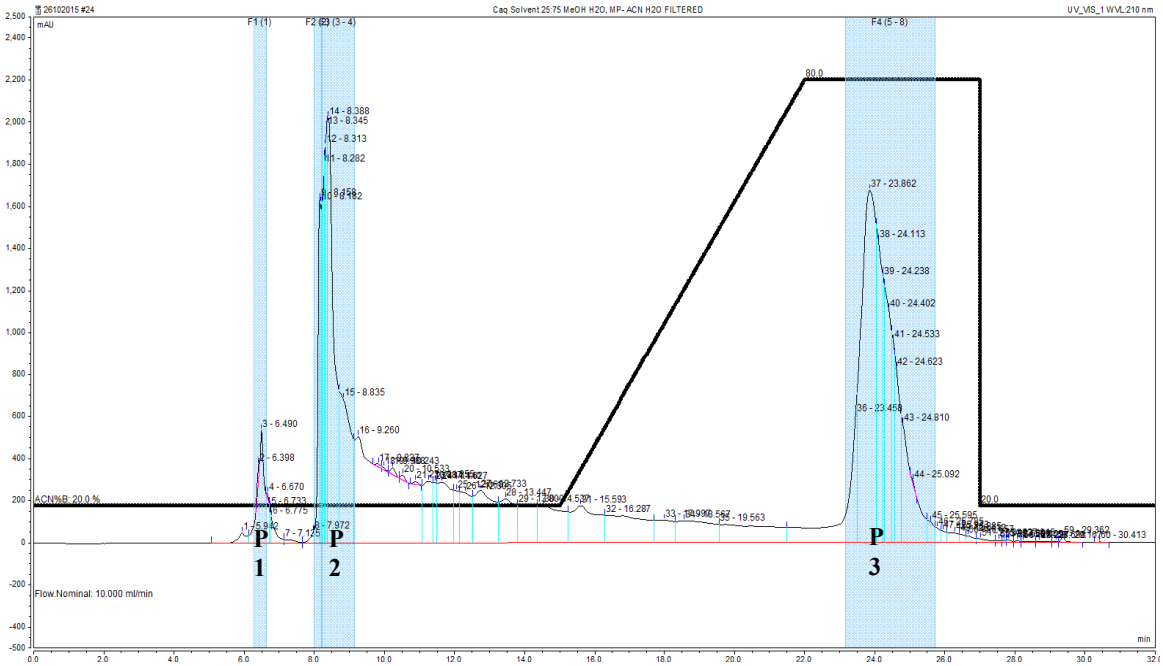
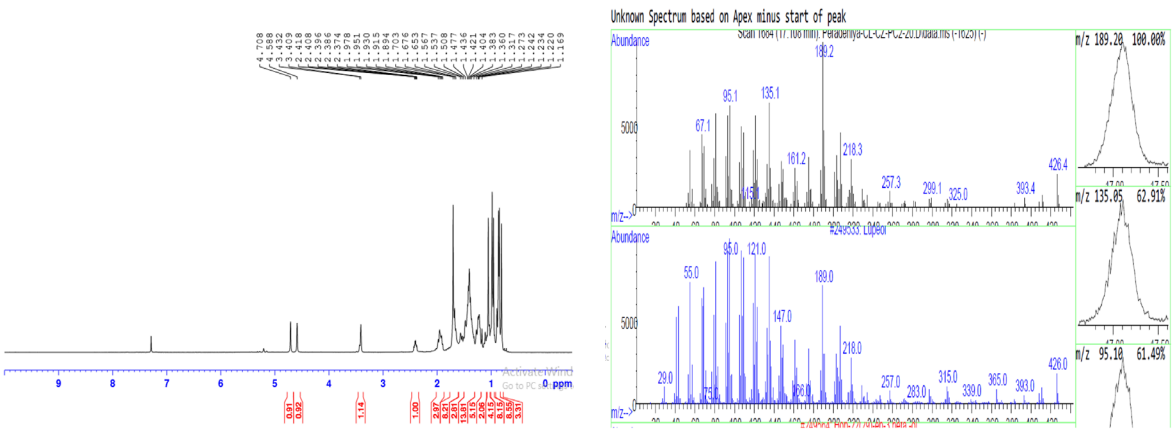


Figure 3. Peaks of unresolved fractions of semipreparative HPLC profile of aqueous fraction (P1, peak 1; P2, peak 2; P3, peak 3)

C. zeylanicum extracts. After 2 and 4 h, there was no significant difference between blood glucose levels in any of the groups and the blood glucose levels were normal. Accordingly, glucose tolerance of rats improved significantly within 1 h by extracts of *C. zeylanicum* bark,



a b

Figure 4. (a), ^{1}H NMR of lupeol; (b), GSMS spectrum and library searched result of lupeol

and after 1 h, the blood glucose levels started to drop towards normal levels. This indicates that *C. zeylanicum* extracts possess good antihyperglycaemic activity. Thus, the use of *C. zeylanicum* bark in diabetic medications may help to improve the glucose tolerance of diabetics.

Phytochemical studies

Phytochemical screening

Phytochemical screening revealed the presence of tannins, phenolic compounds, sterols and flavonoids.

Bioassay guided fractionation

Bioassay guided solvent fractionation indicated that the methanol fraction is highly active compared to dichloromethane fraction. Then the *n*-butanol and water solvent-solvent partitioning of methanol fraction suggested that the aqueous fraction has high *in vitro* antidiabetic activity. Fractionation of aqueous fraction using semi-preparative HPLC gave 3 fractions that could not be further fractioned and identified any compounds. Those unresolved fractions were then subjected to phytochemical screening and the presence of tannins and flavonoids were revealed.

Isolation and identification of chemical compounds responsible for antidiabetic activities

Several compounds were isolated and identified from the OSE of *C. zeylanicum*. However, only lupeol is reported here as it is the compound showing antidiabetic activity. This study reports the first isolation of lupeol (~0.25 %) from *C. zeylanicum*.

Lakshmi *et al*. have shown that lupeol and its derivatives possess significant antidiabetic activities using STZ induced diabetic rats and suggested that lupeol is an inhibitor of glucosidase enzyme [26]. Ramu *et al*. have observed that lupeol reverses the fasting hyperglycaemia, abnormalities in serum/urine protein, urea and creatinine in diabetic rats [27]. In addition, lupeol is an important bioactive molecule exerting other activities [28] in addition to its anti-diabetic properties. Some of the compounds isolated from fractions of OSE of *C. zeylanicum* could not be identified due to the complex nature of their NMR spectra and inadequacy of materials.

Conclusion

Both aqueous and organic extracts of *C. zeylanicum* bark were non-toxic and exhibited α-amylase- and α-glucosidase-inhibitory activity and good hypoglycaemic activity in normoglycaemic rats with marked glucose tolerance. This plant is a potential source for developing effective non-toxic drugs/formulations in controlling diabetes.

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Conflicts of interests

The authors declare that they have no competing financial interests.

References

1. World Health Organisation, Diabetes Fact Sheet No. 312. In: World Health Organization, Media Centre, 2018. https://www.who.int/health-topics/diabetes#tab=tab_1 Accessed July 2020.
2. **Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guarigguata, L., Motala, A.A., Ogurtsova, K., Shaw, J.E., Bright, D. and Williams, R. (2019).** Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation, Diabetes Atlas. Diabetes Res. Clin. Pract. 157: 107843.
3. **Williams, R., Karuranga, S., Malanda, B., Saeedi, P., Basit, A., Besançon, S., Bommer, C., Esteghamati, A., Ogurtsova, K., Zhang, P. and Colagiuri, S. (2020).** Global and regional estimates and projections of diabetes-related health expenditure: Results from the International Diabetes Federation, Diabetes Atlas. Diabetes Res. Clin. Pract. 162(2020): 108072.
4. **Inzucchi, S.E., Bergenstal, R.M., Buse, J.B., Diamant, M., Ferrannini, E., Nauck, M., Peters, A.L., Tsapas, A., Wender, R. and Matthews, D.R. (2012).** Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Spectrum. 25(3): 154-171.
5. World Health Organisation, WHO Traditional Medicines Strategy pp. 2014-2023. WHO, Geneva, Switzerland, 2013.
6. **Rizvi, S.I. and Mishra, N. (2013).** Traditional Indian medicines used for the management of diabetes mellitus. Journal of Diabetes research. 2013: 712092
7. **Kasole, R., Martin, H.D. and Kimiywe, J. (2019).** Traditional medicine and its role in the management of diabetes mellitus: patients' and herbalists' perspectives. J. Evidence-Based Complementary Altern. Med. 2019: 2835691
8. **Dassanayake, M.D. and Clayton, W.D. (1999).** A revised handbook to the flora of Ceylon, vol. 13, National Herbarium, Department of Agriculture, Peradeniya, Sri Lanka.
9. **Ashton, M.S., Gunatilleke, S., de Zoysa, N., Dassanayake, M.D., Gunatilleke N. and Wijesundera, S. (1997).** A Field Guide to the Common Trees and Shrubs of Sri Lanka. WHT Publications (Pvt.) Limited, Sri Lanka. 402-408 p.
10. **Jayaweera, D.M.A. (2006).** Medicinal plants (indigenous and exotic) used in Ceylon: Part 1. The National Science Foundation, Sri Lanka. 207 p.
11. **Ediriweera, E.R.H.S.S. and Ratnasooriya, W.D. (2009).** A review on herbs used in treatment of diabetes mellitus by Sri Lankan ayurvedic and traditional physicians. AYU (An International Quarterly Journal of Research in Ayurveda). 30(4): 373-391.
12. **Arambewela, L.S.R., Arawwawala, L.D.A.M. and Ratnasooriya, W.D. (2005).** Antidiabetic activities of aqueous and ethanolic extracts of *Piper betle* leaves in rats. J. Ethnopharmacol. 102(2): 239-245.
13. National Research Council of the National Academies, 8th edition, The National Academic Press, Washington D.C., USA, 2011
14. **Dissanayake, V.H.W., Gunatilake, M., Chenthuran T. and Jayamanne, N.M.M. (2009).**

Guidelines for Ethics Review of Research Proposals Involving Animals in Sri Lanka. Ethics Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka. 1-8 p.

15. **Nickavar, B., Abolhasani, L. and Izadpanah, H. (2008).** α-Amylase inhibitory activities of six *Salvia* species. Iran. J. Pharm. Res. 7(4): 297-303.
16. **Lakshmanasenthil, S., Vinoth Kumar, T., Geetharamani, D. and Shanthi Priya, S. (2018).** α-Amylase and α-glucosidase inhibitory activity of tetradecanoic acid (TDA) from *Sargassum wightii* with relevance to Type 2 diabetes mellitus. Journal of Biologically Active Products from Nature. 8(3): 180-191.
17. **Sharp, H., Hollinshead, J., Bartholomew, B.B., Oben, J., Watson, A. and Nash, R.J. (2007).** Inhibitory effects of *Cissus quadrangularis* L. derived components on lipase, amylase and alpha-glucosidase activity *in vitro*. Nat. Prod. Commun. 2(8): 817-822.
18. **Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.J. and McLaughlin, J.L. (1982).** Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45(5): 31-34.
19. OECD, OECD Guideline for the Testing of Chemicals, Guideline 423: Acute Oral Toxicity-Acute Toxic Class Method, 2001. https://ntp.niehs.nih.gov›iccvam›supp docs ›feddocs › oecd_gl423
20. **Dhawan, B.N. and Srimal, R.C. (1984).** The Use of Pharmacological Techniques for the Evaluation of Natural Products. In: Proceedings of the Workshop on the Pharmacological Techniques Used for Evaluation of Natural Products. Lucknow: UNESCO. 1982. pp. 88-97.
21. **Taderera, T., Gomo, E. and Shoriwa Chagonda, L. (2016).** The antidiabetic activity of an aqueous root extract of *Annona stenophylla* Engl. and diels in non-diabetic control and alloxan-induced diabetic rats. Journal of Biologically Active Products from Nature. 6(4): 315-322.
22. **Fernando, M.R., Thabrew, M.I. and Karunanayake, E.H. (1990).** Hypoglycaemic activity of some medicinal plants in Sri-Lanka. General Pharmacology: The Vascular System. 21(5): 779-782.
23. **Ugochukwu, S.C., Uche, A. and Ifeanyi, O. (2013).** Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. Asian J. Plant Sci. Res. 3(3): 10-13.
24. **Kumar, S., Narwal, S., Kumar, V. and Prakash, O. (2011).** α-Glucosidase inhibitors from plants: A natural approach to treat diabetes. Pharmacogn. Rev. 5(9): 19-29.
25. **Gupta, M.P., Monge, A., Karikas, G.A., Lopez de Cerain, A., Solis, P.N., De Leon, E., Trujillo, M., Suarez, O., Wilson, F., Montenegro, G., Noriega, Y., Santana, A.I., Correa, M. and Sanchez, C. (1996).** Screening of Panamanian medicinal plants for brine shrimp toxicity, crown gall tumor inhibition, cytotoxicity and DNA intercalation. International J. Pharmacogn. 34(1): 19-27.
26. **Lakshmi, V., Mahdi, A.A., Ahmad, M.K., Agarwal, S.K. and Srivastava, A.K. (2015).** Antidiabetic activity of lupeol and lupeol esters in streptozotocin-induced diabetic rats. Bangladesh Pharm. J. 17(2): 138-146.
27. **Ramu, R., Shirahatti, P.S., Zameer, F., Dhananjaya, B.L. and Prasad, N. (2016).** Assessment of *in vivo* antidiabetic properties of umbelliferone and lupeol constituents of banana (*Musa* sp. var. Nanjangud Rasa Bale) flower in hyperglycaemic rodent model. PLoS one. 11(3): e0151135.
28. **Soni, R., Nahata, A., Jain, A., Singhai, A.K. (2014).** Antihepatotoxic potential of barks of *Crataeva nurvala: in vitro* and *in vivo* studies. Journal of Biologically Active Products from Nature. 4(2): 101-110.