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Phytochemical composition, antioxidant capacity, and antidiabetic potential of various leaf extracts of *Garcinia lanceifolia* Roxb.

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ABSTRACT

Introduction: *Garcinia lanceifolia* Roxb. has been used to manage metabolic disorders such as diabetes. This study investigated the impacts of aqueous and graded ethanolic extracts on the phytochemical composition, antioxidant capacity, and antidiabetic efficacy of *G. lanceifolia* leaves.

Methods: Leaves were extracted via aqueous decoction and ethanol maceration at 25%, 50%, 75%, and 95% concentrations. Qualitative screening identified phytochemicals, while total phenolic content (TPC) and total flavonoid content (TFC) were quantified. Significant bioactive components were examined utilizing gas chromatography-mass spectroscopy (GC-MS). The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. *In vitro* α-glucosidase and α-amylase inhibition assays were conducted.

Results: Alkaloids, saponins, and terpenoids were consistently present, while tannins and steroids appeared in 75-95% ethanol extracts. The 95% ethanol extract showed the highest TPC (20.62 ± 0.61 mg gallic acid equivalent/g) and TFC (65.92 ± 1.76 mg quercetin equivalent/g), and GC-MS identified multiple pharmacologically active compounds. This extract exhibited the strongest antioxidant activity in DPPH (379.07 ± 1.33 mg Trolox equivalent/g) and ABTS (12.56 ± 1.00 mg Trolox equivalent/g) assays. Enzyme inhibition was concentration-dependent, with IC_{50} values of 0.533 ± 0.098 mg/mL for α-glucosidase and 0.286 ± 0.031 mg/mL for α-amylase.

Conclusion: Extraction with high-concentration ethanol enhanced the recovery of bioactive compounds, resulting in superior antioxidant and antidiabetic activities. These findings support its traditional use and highlight ethanolic extracts as potential natural agents for managing diabetes and oxidative stress. Further studies should isolate individual compounds and evaluate their *in vivo* efficacy.

Implication for health policy/practice/research/medical education:
The strong antioxidant and antidiabetic activities of ethanolic *G. lanceifolia* leaf extracts indicate their potential as natural therapeutic agents for managing diabetes and oxidative stress. These findings highlight the need for further research to isolate individual bioactive compounds and assess their *in vivo* efficacy, and may inform health policy, clinical practice, and medical education on the use of plant-based interventions.

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Introduction

Type 2 diabetes mellitus (T2DM), a major subtype of diabetes, continues to pose a serious global health challenge, primarily characterized by chronic hyperglycemia resulting from insulin resistance or insufficient insulin secretion. A critical strategy in controlling postprandial hyperglycemia involves inhibiting carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase, which are responsible for carbohydrate digestion and glucose absorption (1). While synthetic antidiabetic agents such as sulfonylureas, metformin, voglibose, and acarbose are widely prescribed and effective, their use is frequently associated with adverse effects, including gastrointestinal discomfort, renal complications, lactic acidosis, and multi-organ failure (2). Growing evidence suggests that chronic hyperglycemia contributes to the overproduction of oxygen-derived free radicals and nitrogen radicals, which are key drivers of diabetes-related oxidative damage (3). As a result, recent research has turned to natural compounds with potent antioxidant properties to help neutralize oxidative stress and slow the progression of T2DM (4). Compared to conventional medications, natural substances generally offer improved biocompatibility and a lower risk of side effects. Many plant-derived compounds also exhibit significant α-amylase and α-glucosidase inhibitory activity, further enhancing their antidiabetic potential (5). Notably, secondary metabolites such as alkaloids, terpenoids, steroids, and tannins have demonstrated both antioxidant and antidiabetic effects in various medicinal plants (6,7). Thus, identifying plants that offer dual therapeutic benefits, combating oxidative stress while modulating glucose metabolism, remains a key research priority.

The genus *Garcinia* (family: *Clusiaceae*) comprises over 300 species predominantly found in tropical rainforests in Asian countries such as China, India, Vietnam, Indonesia, Malaysia, and Thailand (8). In Thailand, twenty-nine species have been documented (9). One of these species, *Garcinia lanceifolia* Roxb. (syn. *Garcinia gracillis* Pierre), locally known as Cha-mang or Mak-paem, produces edible red fruits and green leaves that have been traditionally used as flavoring agents in local cuisine (10). Various parts of *G. lanceifolia* have a broad spectrum of bioactive chemicals that are responsible for their pharmacological properties. The acidic fruit contains notable constituents such as xanthones, bioflavonoids, benzophenones, and triterpenes, which are reported to have antimicrobial, antidiarrheal, antidysenteric, antipyretic, and hepato-protective properties (11,12). The stem bark exhibits antidiabetic and antiulcer activity (13), while the roots are traditionally used in folk remedies as antipyretics (14). Additionally, studies on the whole plant have revealed antinociceptive, antihyperglycemic, and membrane-stabilizing effects (15). Methanolic extracts from the leaves of *G. lanceifolia* have demonstrated promising biological activities, including superoxide scavenging, DNA protection, and neuroprotection (16). However, methanol is not suitable for food or pharmaceutical use due to its toxicity. In contrast, ethanol and water are considered non-toxic, food-grade solvents that are both safe and environmentally sustainable, making them preferable for extracting bioactive compounds intended for human applications (17). Despite these advantages, there is still limited research focused on the chemical constituents and physiological efficacy of *G. lanceifolia* leaf extracts obtained using these safer solvent systems.

To the best of our knowledge, there is no comprehensive study that has evaluated the phytochemical profile and biological functions of *G. lanceifolia* leaf extracts using food-grade solvents with different extraction techniques. This study represents the first report to investigate this combination, offering new insights for the potential development of *G. lanceifolia* as a natural therapeutic candidate for T2DM. To address this gap, the present research examined the phytochemical profiles and bioactivities of leaf extracts obtained via maceration and decoction using ethanol and water as solvents. Specifically, the contents of phenolic and flavonoid compounds were quantified along with the antioxidant activity. Moreover, the inhibitory effects on the carbohydrate-hydrolizing enzymes (α-glucosidase and α-amylase enzymes) were assessed. This comprehensive investigation highlights the medicinal potential of *G. lanceifolia* and encourages its continued exploration as a natural remedy for diabetes and disorders linked to oxidative stress.

Materials and Methods

Chemicals

Ethanol, acarbose, Folin-Ciocalteu reagent, sodium carbonate, sodium hydroxide, sodium acetate, gallic acid, aluminum chloride, phosphate buffer, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), *p*-nitrophenol glucopyranoside (*p*NPG), α-Glucosidase from *Saccharomyces cerevisiae*, and porcine pancreatic α-amylase were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All of the chemicals were of analytical grade.

Plant materials

In January 2025, the leaves of *G. lanceifolia* were collected from the Khao Hin Sorn Royal Development Study Center area (13.74465° N, 101.50865° E) in Chachoengsao Province, Thailand. Plant identification was carried out by Dr. Boonmee Phokham, a taxonomist at Abhaibhubejhr College of Thai Traditional Medicine, Prachinburi, Thailand, and verified based on morphological characteristics. Voucher specimens were deposited in the Herbarium of Abhaibhubejhr College of Thai Traditional Medicine, Faculty of Public Health and Allied Health

Sciences, Praboromarajchanok Institute, under the accession number 27062025-01. The collected leaves were thoroughly washed, oven-dried at 50 °C for 48 hours using a hot air oven (Binder FD53, Germany), and ground into a fine powder at 22,000 rpm with an electric grinder (Waring 8011BU, USA). The samples were kept frozen (-20 °C) in sealed containers.

Preparation of crude extracts

The crude extracts of *G. lanceifolia* were prepared using decoction and maceration methods, modified from Puangploy et al (18). For the aqueous extract, 100 g of dried leaf powder was decocted with 700 mL of distilled water at 60 °C for 20 minutes. For ethanol-based extracts, maceration of dried leaf powder (100 g) was carried out with 500 mL of ethanol at varying concentrations (25%, 50%, 75%, and 95%) at ambient temperature for seven days. The solutions were filtered (Whatman No. 1), concentrated using a rotary evaporator at 50 °C (Buchi R205, Switzerland), and further dried in a hot air oven at 50°C for 48 hours (Binder FD53, Germany). The extracts were preserved under frozen (–20 °C) conditions in airtight containers for the next experiment.

Phytochemical analysis

Preliminary phytochemical screening

Preliminary phytochemical screening of the crude extract was conducted using standard qualitative methods. Alkaloids were identified by Wagner's test, flavonoids by Shibita's reaction test, anthraquinones by Borntrager's reaction, and coumarins by the NaOH test. Saponins were detected using the frothing test, tannins by the ferric chloride test, steroids by the Liebermann-Burchard test, terpenoids by the Salkowski test, and cardiac glycosides by the Keller-Killani test (18).

Total phenolic content (TPC)

The extract was analyzed based on the procedure described by Tohkubaha et al (19). A crude extract (10 mg) was dissolved in 5 mL of 80% ethanol (2 mg/mL). An aliquot was reacted with Folin–Ciocalteu reagent and sodium carbonate, kept at room temperature (30 minutes), and the spectrophotometric measurements were carried out at 765 nm (BMG Labtech, Germany). TPC was calculated from a gallic acid calibration curve.

Total flavonoid content (TFC)

According to the method reported by Chatatikun et al (20), a crude leaf extract of *G. lanceifolia* (10 mg) was dissolved in 10 mL of 95% ethanol (1 mg/mL). In a 96-well plate, the extract (50 µL) was mixed with ethanol, sodium acetate, and aluminum chloride. After incubation (40 minutes at ambient temperature under dark conditions), the spectrophotometric measurements were carried out at 415 nm (BMG Labtech, Germany). The TFC was determined from a quercetin calibration curve.

Gas chromatography-mass spectroscopy (GC-MS) analysis

The phytochemical profile of the crude extracts was analyzed by GC-MS at the Center for Instrumentation and Analytical Testing, Faculty of Science, Energy and Environment, King Mongkut's University of Technology North Bangkok, Rayong Campus, using an Agilent 7890B GC coupled with a 5977B MSD (Agilent Technologies, USA) and controlled by MassHunter Software. Separation was achieved on an HP-5ms Ultra Inert column (30 m × 250 µm i.d., 0.25 µm film) with helium as the carrier gas (1 mL/min). The injector was maintained at 300 °C, with 1 µL injected in split mode. The oven program was 50 °C (initial), ramped at 10 °C/min to 310°C, and held for 10 minutes. The MS operated in electron ionization mode (70 eV) with a multiplier voltage of 1200 V, ion source at 230 °C, quadrupole at 150 °C, solvent delay of 4 minutes, and scan range of m/z 50–700. Compounds were identified by comparing retention times and mass spectra with NIST library data. The methodology was modified from Limcharoen et al. (21).

Antioxidant activities

DPPH radical scavenging assay

According to the method of Singsai et al (22), a crude leaf extract of *G. lanceifolia* (0.01 g) was dissolved in 10 mL of 10% DMSO. The extract (100 µL) was mixed with 0.2 mM DPPH in methanol (100 µL). After incubation (30 minutes, under dark conditions), the spectrophotometric measurements were carried out at 517 nm (BMG Labtech, Germany). A Trolox calibration curve was applied for the antioxidant activity determination.

ABTS radical scavenging assay

The method described by Wanna et al (23) was followed. $ABTS^{+}$ stock solution was prepared by mixing 4.0 mM ABTS with 2.45 mM potassium persulfate (1:1) and incubating for 12 hours in the dark. The solution was adjusted with distilled water until its absorbance reached 0.9 ± 0.05 at 734 nm. Twenty microliters of the extract (20 mg/mL) were mixed with ABTS working solution (150 µL). After incubation (15 minutes, under dark conditions), the spectrophotometric measurements were performed at 734 nm (BMG Labtech, Germany). Antioxidant activity was determined from a Trolox calibration curve.

In vitro antidiabetic activity

α-Glucosidase inhibition activity

A modified method of Matsui et al (24) was carried out. After adding 20 µL of extracts at different concentrations to the phosphate buffer with a pH of 6.8 (120 µL of 10 mM), 20 µL of α-glucosidase (0.33 U/mL) was added. For 15 minutes, the mixtures were incubated at 25 °C. After adding 20 µL of 5 mM *p*NPG to the reaction mixture, it

was allowed to incubate for 15 minutes at 25 °C. Later, 40 µL of a 1 mM sodium carbonate solution was added to stop the reaction. At 405 nm, the mixture's absorbance was measured by a microplate reader (BMG Labtech, Germany). Acarbose solutions (0.31–40 mg/mL) were used as a positive control. The percent inhibition value was obtained based on the equation given below, where A is the absorbance of the buffer + *p*NPG + enzyme mixture; B is that of the buffer–*p*NPG mixture, C represents the buffer–*p*NPG–enzyme solution, and D represents the buffer–*p*NPG solution lacking enzyme.

$$\% \text{ Inhibition} = \{[(A-B)-(C-D)]/(A-B)\} \times 100$$

α-Amylase inhibition activity

Utilizing a modified approach based on Sangchan et al (25), the α-amylase inhibitory activity was investigated. After being weighed at 400 mg, leaf extracts of *G. lanceifolia* were diluted in 10 mL of 99% (v/v) methanol, obtaining a final extract concentration of 40 mg/mL. The assay started with the addition of 400 µL of porcine pancreatic α-amylase (10 U/mg), 1,200 µL of the plant extract solution, and 2.5 mL of 20 mM potassium phosphate buffer (pH 7.0) to a test tube. The mixture was incubated at 37 °C for five minutes. The mixture was incubated for an additional five minutes at room temperature after adding 4 mL of starch solution (0.35 g/L) to the test tube. After 2, 4, 6, 8, and 10 minutes, the reaction mixture (1 mL) was mixed instantly with 5 mM iodine solution (1 mL). At 620 nm, the absorbance was determined with a spectrophotometer (BMG Labtech, Germany). The usage of acarbose worked as a positive control. Using the following formula, the α-amylase inhibition activity was determined:

$$\% \text{ Inhibition} = [Vi_{control} - Vi_{sample}]/ Vi_{control} \times 100$$

The difference between the reaction rates of the enzyme without extract ($Vi_{control}$) and the enzyme with extract (Vi_{sample}) was used to determine the percentage of inhibition (% inhibition), which indicated the decrease in enzymatic activity. The average slope of at least five data points that show a linear trend with an R^2 value higher than 0.95 was used to calculate the enzymatic reaction rate. The IC_{50} value was determined according to Apostolidis et al (26).

Statistical analysis

All data were expressed as mean ± standard deviation from three independent replicates, and were statistically analyzed using analysis of variance (ANOVA) in IBM SPSS Statistics 25.0. For determining differences between the means, Duncan's new multiple range test (DMRT) was used, with a significance level of $P < 0.05$.

Results

Phytochemical analysis

Phytochemical screening and extraction yield

The extraction yields (% w/w) of various solvent extracts from *G. lanceifolia* leaves are listed in Table 1. The highest yield was obtained using the decoction method with distilled water, whereas maceration with ethanol at different concentrations produced lower yields. Interestingly, higher ethanol concentrations (75% and 95%) resulted in greater extraction yields compared to lower concentrations (25% and 50%), suggesting that a higher proportion of ethanol improves extraction efficiency. All extracts consistently contained alkaloids, saponins, and terpenoids, as confirmed by phytochemical screening, whereas tannins and steroids were selectively detected in ethanol-based extracts (Table 2). Notably, the 95% ethanol extract exhibited the broadest phytochemical diversity, containing alkaloids, saponins, terpenoids, tannins, and steroids. These compounds likely contribute to the extract's strong antioxidant and antidiabetic activities, suggesting its potential for further pharmacological investigation.

Evaluation of total phenolic and flavonoid contents

The total phenolic and flavonoid contents of *G. lanceifolia* leaf extracts, which varied depending on the type and polarity of the solvent used, were calculated from a gallic acid calibration curve (20–100 µg/mL; $y = 0.0052x + 0.0948$; $R^2 = 0.9992$) and a quercetin calibration curve (50–250 µg/mL; $y = 0.0026x + 0.0306$; $R^2 = 0.9999$), respectively (Table 3). Overall, the contents increased with higher ethanol concentrations, reaching the highest levels in the 95% ethanol extract, whereas the aqueous extract exhibited the lowest values. These results indicate that solvent polarity strongly influences the extraction efficiency of phenolic and flavonoid compounds.

Table 1. The extraction yield (%) of different solvents from the leaves of *Garcinia lanceifolia*

Solvents	Extract methods	Yield (%w/w)
Aqueous	Decoction	20.31±1.37[a]
25% Ethanol	Maceration	9.87±1.19[c]
50% Ethanol	Maceration	9.52±1.81[c]
75% Ethanol	Maceration	15.73±1.05[b]
95% Ethanol	Maceration	14.58±1.60[b]

Data are presented as the average value with standard deviation. Values followed by different letters within a column are significantly different ($P < 0.05$).

Table 2. Phytochemical screening of different solvents from the leaves of *Garcinia lanceifolia*

Phytochemical compounds	Decoction aqueous	Maceration			
		25% Ethanol	50% Ethanol	75% Ethanol	95% Ethanol
Alkaloids	+	+	+	+	+
Flavonoids	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Coumarins	-	-	-	-	-
Saponins	++	++	++	++	++
Tannins	-	-	+	+	+
Steroids	-	-	-	+	++
Terpenoids	+	+	+	++	++
Cardiac glycoside	-	-	-	-	-

The presence of chemical constituents is: (-) absent, (+) low concentration, (++) high concentration.

Table 3. Total of phenolic and flavonoid contents in different solvent extracts from *Garcinia lanceifolia* leaves

Extracts	Total phenolic content(mg GAE/g crude extract)	Total flavonoid content (mg QE/g crude extract)
Aqueous	10.37±0.67^{d}	31.18±1.97^{d}
25% Ethanol	13.42±0.54^{c}	32.72±2.12^{d}
50% Ethanol	16.11±1.46^{b}	37.59±1.55^{c}
75% Ethanol	20.21±0.97^{a}	49.77±2.77^{b}
95% Ethanol	20.62±0.61^{a}	65.92±1.76^{a}

Data are presented as the average value with standard deviation. Different superscript letters in the same column denote statistically significant differences at $P < 0.05$. Abbreviations: mg GAE/g crude extract: gallic acid equivalent milligrams per gram of crude extract; mg QE/g crude extract: quercetin equivalent in milligrams per gram of crude extract.

Identified chemical constituents

GC-MS analysis was conducted to characterize the chemical constituents of various solvent extracts from *G. lanceifolia* leaves (Figure 1). Compound identification was based on a similarity index exceeding 80% against the NIST spectral library. A total of 5 compounds were detected in the aqueous extract, 4 compounds in 25% ethanol, 6 compounds in 50% ethanol, 7 compounds in 75% ethanol

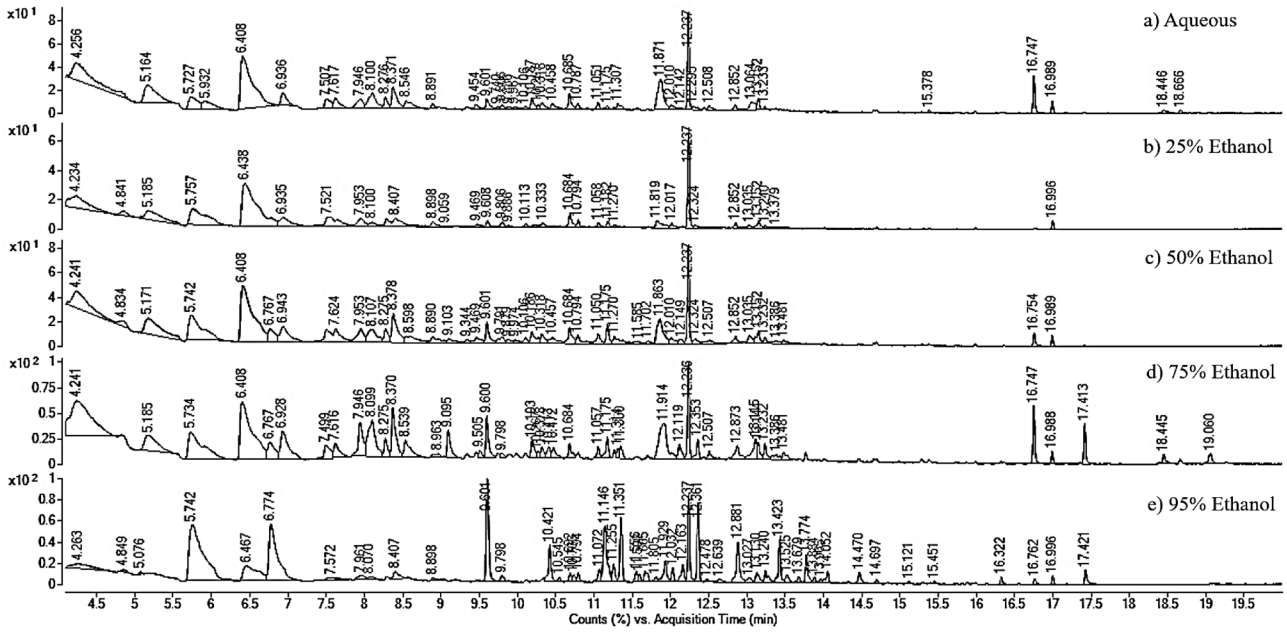

Figure 1. Comparison of gas chromatography-mass spectroscopy chromatograms for *Garcinia lanceifolia* leaf extracts prepared with different solvents. a) Aqueous, b) 25% ethanol, c) 50% ethanol, d) 75% ethanol, and e) 95% ethanol.

extracts, and 18 compounds in 95% ethanol extract, as summarized in Table 4. Certain compounds, including butanedioic acid, dimethyl ester (dimethyl succinate), 2,5-furandicarboxaldehyde (2,5-diformylfuran), 2,4-di-tert-butylphenol (2,4-DTBP), and benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (Metilox), were present across all extracts, while hexadecanoic acid, methyl ester (methyl palmitate), was

Table 4. Compounds identified in *Garcinia lanceifolia* extracts by gas chromatography-mass spectroscopy

Peak	RT (min)	Component names (Reference with the library)	Molecular formula	Molecular weight (g mol^{-1})	Peak area (%)	Similarity index (%)
Aqueous extract						
1.	5.727	Butanedioic acid, dimethyl ester	$C_6H_{10}O_4$	146.1	3.78	82
2.	6.408	2,5-Furandicarboxaldehyde	$C_6H_4O_3$	124.0	20.80	91
3.	12.237	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.2	5.89	90
4.	16.747	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.3	2.43	87
5.	16.989	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292.2	0.73	91
25% Ethanol extract						
1.	5.757	Butanedioic acid, dimethyl ester	$C_6H_{10}O_4$	146.1	10.41	80
2.	6.438	2,5-Furandicarboxaldehyde	$C_6H_4O_3$	124.0	25.79	87
3.	12.237	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.2	7.52	90
4.	16.996	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292.2	0.60	91
50% Ethanol extract						
1.	5.742	Butanedioic acid, dimethyl ester	$C_6H_{10}O_4$	146.1	8.92	82
2.	6.408	2,5-Furandicarboxaldehyde	$C_6H_4O_3$	124.0	20.69	90
3.	9.601	Trimethyl-1,1,2-ethanetricarboxylate	$C_8H_{12}O_6$	204.1	1.62	80
4.	12.237	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.2	5.74	90
5.	16.754	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.3	0.89	86
6.	16.989	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292.2	0.57	92
75% Ethanol extract						
1.	5.734	Butanedioic acid, dimethyl ester	$C_6H_{10}O_4$	146.1	7.50	80
2.	6.408	2,5-Furandicarboxaldehyde	$C_6H_4O_3$	124.0	13.15	89
3.	9.600	Trimethyl-1,1,2-ethanetricarboxylate	$C_8H_{12}O_6$	204.1	2.24	83
4.	12.236	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.2	3.42	91
5.	16.747	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.3	2.15	88
6.	16.989	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292.2	0.42	91
7.	17.413	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.3	1.47	90
95% Ethanol extract						
1.	5.742	Butanedioic acid, dimethyl ester	$C_6H_{10}O_4$	146.1	19.65	94
2.	6.467	2,5-Furandicarboxaldehyde	$C_6H_4O_3$	124.0	5.41	80
3.	6.774	Butanedioic acid, ethyl methyl ester	$C_7H_{12}O_4$	160.1	12.28	83
4.	9.601	Trimethyl-1,1,2-ethanetricarboxylate	$C_8H_{12}O_6$	204.1	7.13	91
5.	11.146	1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	$C_{15}H_{24}$	204.2	5.63	91
6.	11.255	3-Isopropyl-6,8a-dimethyl-1,2,4,5,8,8a-hexahydroazulene	$C_{15}H_{24}$	204.2	1.25	86
7.	11.351	Bicyclosesquiphellandrene	$C_{15}H_{24}$	204.2	3.89	89
8.	11.556	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1.alpha.,4.alpha.,7.alpha.)]-	$C_{15}H_{24}$	204.2	0.65	86
9.	11.592	Thujopsene-I3	$C_{15}H_{24}$	204.2	0.42	85
10.	11.665	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-, (1R,2S,7R,8R)-	$C_{15}H_{24}$	204.2	1.02	89
11.	12.237	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.2	4.40	89
12.	12.361	(1R,5S)-1,8-Dimethyl-4-(propan-2-ylidene)spiro[4.5]dec-7-ene	$C_{15}H_{24}$	204.2	3.92	92
13.	13.423	Caryophyllenyl alcohol	$C_{15}H_{26}O$	222.2	2.63	83
14.	14.470	(3S,3aS,5R,6S,7aS)-3,6,7,7-Tetramethyloctahydro-3a,6-ethanoinden-5-ol	$C_{15}H_{26}O$	222.2	0.95	83
15.	16.322	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.3	0.37	83
16.	16.762	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.3	0.35	83
17.	16.996	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292.2	0.36	91
18.	17.421	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.3	1.08	86

RT: Retention time.

absent only in the 25% ethanol extract. The high number of bioactive compounds in the 95% ethanol extract likely contributes to its superior antioxidant and antidiabetic activities observed in DPPH, ABTS, α-glucosidase, and α-amylase assays. Compounds such as 2,4-DTBP and dimethyl succinate are known for their radical-scavenging properties, while fatty acid esters like methyl palmitate may play a role in enzyme inhibition, collectively supporting the pharmacological potential of the 95% ethanol extract.

Antioxidant activity

The antioxidant properties of *G. lanceifolia* leaf extracts were determined using the DPPH and ABTS assays, calculated from Trolox calibration curves (DPPH: 200-1,000 µg/mL; $y = -0.0003x + 0.7136$; $R^2 = 0.9901$; ABTS: 10-80 µg/mL; $y = -0.005x + 0.5678$; $R^2 = 0.9926$). As shown in Table 5, the antioxidant activity increased with ethanol concentration, with the 95% ethanol extract exhibiting the strongest radical scavenging capacity and the aqueous extract the weakest. This trend corresponds with the phytochemical composition of the extracts, suggesting that solvent polarity significantly affects the extraction of bioactive compounds, particularly phenolics and flavonoids, which largely contribute to the observed free radical-neutralizing activity.

In vitro antidiabetic activity

The *in vitro* antidiabetic activity of *G. lanceifolia* leaf extracts prepared using different solvents was assessed. The IC_{50} values were calculated for each extract and compared with acarbose, a standard antidiabetic drug used as a positive control. As shown in Table 6, the ethanolic extracts demonstrated stronger inhibitory effects on both α-glucosidase and α-amylase compared to the aqueous extract, with 95% ethanol being the most effective. In particular, the 95% ethanol extract demonstrated the greatest α-glucosidase inhibition, while both 75% and 95% ethanol extracts were highly active against α-amylase. Although all extracts were less potent than the standard drug acarbose, the results highlight that ethanol-rich solvents, especially 95% ethanol, enhance the recovery of antidiabetic constituents from *G. lanceifolia* leaves.

Discussion

The extraction yield and phytochemical composition of *G. lanceifolia* leaf extracts were strongly influenced by solvent polarity and extraction method (Tables 1 and 2). The highest yield was achieved through aqueous decoction, which aligns with prior studies suggesting that water, due to its high polarity, effectively extracts polar constituents such as alkaloids, saponins, and certain terpenoids (27). Although aqueous decoction produced the highest crude yield, ethanol-based solvents, particularly at higher concentrations, were more effective in recovering diverse bioactive constituents. All extracts consistently contained alkaloids, saponins, and terpenoids, as confirmed by phytochemical screening, while tannins and steroids were detected only in ethanol-rich fractions. This selective

Table 5. Antioxidant activity in different solvent extracts from *Garcinia lanceifolia* leaves determined by DPPH and ABTS assays

Extracts	DPPH (mg TEAC/g crude extract)	ABTS (mg TEAC/g crude extract)
Aqueous	270.62±1.39^{e}	3.08 ± 0.07^{d}
25% Ethanol	286.40±1.76^{d}	3.43 ± 0.03^{c}
50% Ethanol	347.07±0.00^{c}	7.54 ± 0.09^{b}
75% Ethanol	373.29±1.92^{b}	11.36 ± 0.92^{a}
95% Ethanol	379.07±1.33^{a}	12.56 ± 1.00^{a}

Data are expressed as mean ± standard deviation. Different superscript letters within the same column indicate significant differences at ($P < 0.05$). DPPH: 2,2- Diphenyl -1- picrylhydrazyl; ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline -6- sulfonic acid); TEAC: Trolox equivalent antioxidant capacity

Table 6. Enzyme inhibition of *Garcinia lanceifolia* leaf extracts against α-glucosidase and α-amylase

Extracts	α-Glucosidase inhibition (IC_{50}) (mg/mL)	α-Amylase inhibition (IC_{50}) (mg/mL)
Aqueous	1.396±0.085^{d}	0.650±0.090^{c}
25% Ethanol	1.293±0.025^{cd}	0.440±0.01^{2}b
50% Ethanol	1.143±0.181^{c}	0.366±0.031^{ab}
75% Ethanol	0.776±0.070^{b}	0.281±0.084^{a}
95% Ethanol	0.533±0.098^{a}	0.286±0.031^{a}
Acarbose	0.226±0.013	0.070±0.002

Data are expressed as mean ± standard deviation. Different superscript letters within the same column indicate significant differences at ($P < 0.05$). IC_{50}: Half-maximal inhibition concentration.

detection likely results from the limited solubility of tannins and steroids in water, necessitating the use of less polar solvents such as ethanol for efficient recovery (28). These results agree with findings from Chowdhury et al (11) and Policegoudra et al (29), who also reported saponins in aqueous extracts of *G. lanceifolia* leaves. Similarly, the detection of alkaloids in all samples supports observations by Ghosh et al (15). Notably, none of the tested extracts contained detectable levels of flavonoids, anthraquinones, coumarins, and cardiac glycosides. This absence could be due to several reasons, including the inherent phytochemical profile of *G. lanceifolia*, limitations in the sensitivity of the qualitative methods applied, or the degradation of these compounds during the extraction process. Another possibility is that these constituents are present only in trace amounts, below the detection limits of the current analytical techniques. Among all extraction conditions tested, the 95% ethanol extract displayed the most diverse phytochemical profile, comprising alkaloids, saponins, terpenoids, tannins, and steroids. This indicates that high-concentration ethanol is particularly effective in extracting a wide range of phytochemicals, encompassing both polar and semi-polar constituents such as phenolics, phospholipids, and lipoprotein-associated compounds, thereby providing a more comprehensive chemical representation than water or lower ethanol concentrations (30,31). The identified phytoconstituents are associated with a wide range of pharmacological properties. For example, alkaloids have been reported to possess antimicrobial and neuroactive effects (32,33); saponins are known for their anti-inflammatory and cholesterol-lowering effects (34,35); terpenoids have been extensively studied for their diverse biological activities such as anti-inflammatory, antioxidant, and anticancer effects (36,37); tannins exhibit antibacterial, antiviral, antidiarrheal, and antitumor properties (38,39); and steroids are recognized for their roles in antidiabetic, immunosuppressive, and antibacterial therapies (40,41). In conclusion, the existence of these bioactive metabolites, particularly in the 95% ethanol extract, highlights the promising therapeutic potential of *G. lanceifolia* leaves and supports their further investigation for pharmacological applications.

Among the phytochemicals, phenolic compounds and flavonoids represent the most abundant and biologically relevant groups; therefore, their total contents were quantified. Based on the findings presented in Table 3, the effectiveness of extracting phenolic and flavonoid compounds from *G. lanceifolia* leaves depends largely on the polarity of the solvent used. The 95% ethanol extract contained the highest levels of phenolics and flavonoids compared with other extracts. This finding contrasts with the extraction yield shown in Table 1, where the aqueous extract produced a higher crude mass than the 95% ethanol extract. Such a discrepancy arises because the extraction yield reflects the total crude mass, including sugars, proteins, chlorophyll, and other non-phenolic constituents, whereas total phenolic and flavonoid assays specifically quantify these bioactive groups (42). Such enhancement in extraction performance is probably a result of the intermediate polarity of 95% ethanol, which enables effective solubilization of a wide spectrum of phytochemicals, including both polar and semi-polar phenolic and flavonoid constituents. Additionally, by breaking down plant cell walls, ethanol promotes the liberation of bioactive compounds. (27). In contrast, the aqueous extract of *G. lanceifolia* produced the lowest levels of both phenolics (10.37 ± 0.67 mg GAE/g crude extract) and flavonoids (31.18 ± 1.97 mg QE/g crude extract), indicating water's limited efficiency for recovering less polar phytochemicals. As a highly polar solvent, water predominantly extracts hydrophilic compounds and is less effective for phenolics and flavonoids that are moderately non-polar or tightly bound within plant matrices. These results are consistent with earlier research highlighting the superiority of hydroalcoholic mixtures over pure water in extracting polyphenolic compounds (30). Similarly, aqueous extractions of *G. lanceifolia* have previously yielded phenolic contents below 200 mg GAE/100 g dry extract, further supporting these observations (29). Interestingly, as shown in Table 2, the TFC was higher than the TPC in all samples. This finding is unexpected, since flavonoids are a subclass of phenolic compounds and, therefore, their levels are generally expected to be lower than the TPC. Although the aluminum chloride method is widely recognized for flavonoid analysis, it has been reported that certain non-flavonoid compounds can interfere with complex formation, potentially leading to false-positive results (43,44). Therefore, complementary analytical approaches are recommended following total flavonoid determination.

The GC-MS profile offers a qualitative insight into the specific phytochemical constituents, together giving a more comprehensive understanding of the extract's composition (45). GC-MS profiling (Figure 1 and Table 4) revealed that 95% ethanol yielded the greatest chemical diversity, identifying 18 unique compounds. This observation is consistent with earlier studies indicating that solvents with intermediate polarity, such as high-concentration ethanol, effectively extract both polar and non-polar phytochemicals from plant materials (46,47). The improved extraction performance of 95% ethanol is presumed to arise from the increased solubility of biologically reacting components in ethanol–water mixtures (48). Certain metabolites, including dimethyl succinate, 2,5-diformylfuran, 2,4-di-tert-butylphenol, and methylated benzenepropanoic acid derivatives, were consistently found across all extracts, suggesting their abundance and stability irrespective of solvent polarity. These compounds are recognized for their diverse pharmacological properties. For example, dimethyl

succinate has been shown to modulate inflammation as well as mitochondrial and endothelial functions (49). Similarly, 2,5-diformylfuran, 2,4-di-tert-butylphenol, and methylated benzenepropanoic acid derivatives are established antioxidants, antifungal, and antimicrobial agents present in various plant species (50,51). Notably, the 95% ethanol extract also contained pharmacologically important compounds such as caryophyllenyl alcohol, hexadecanoic acid esters (methyl and ethyl), and bicyclosesquiphellandrene, which have been linked to antioxidant, anti-inflammatory, antidiabetic, anticancer, antimicrobial, cholesterol-lowering, and 5-alpha reductase inhibitory activities (52-55). The absence of methyl palmitate (hexadecanoic acid methyl ester) in the 25% ethanol extract suggests that the extraction of moderately hydrophobic compounds is highly dependent on solvent polarity. Since methyl palmitate is more soluble in less polar solvents, it was detected in all extracts except the highly polar 25% ethanol sample (56). These findings highlight the critical role of solvent choice in optimizing the recovery of bioactive compounds. The diverse phytochemical profile obtained with 95% ethanol confirms its effectiveness for isolating pharmacologically valuable constituents from *G. lanceifolia* leaves. Future research should focus on bioactivity-guided fractionation using this solvent to purify and characterize individual compounds and assess their therapeutic potential. Moreover, employing advanced extraction methods such as ultrasonic or microwave-assisted extraction may further enhance yield and efficiency (57).

Antioxidants play a crucial role in neutralizing free radicals, thereby preventing cellular damage and disrupting detrimental chain reactions. Among the numerous analytical methods available, the DPPH and ABTS assays are particularly favored due to their simplicity, reliability, and compatibility with standard spectrophotometric equipment, making them widely applicable in laboratory settings (18). While both assays rely on radical scavenging mechanisms, they differ in solubility and radical specificity. DPPH is mainly suited for evaluating lipophilic radicals in organic environments, whereas ABTS is effective in both aqueous and lipid systems and can detect a wider range of hydrophilic and lipophilic antioxidants. Utilizing both assays therefore provides a broader and more reliable evaluation of the antioxidant properties of *G. lanceifolia* leaf extracts (18). The antioxidant capacity of *G. lanceifolia* leaf extracts was significantly influenced by the choice of extraction solvent (Table 4). The extract prepared using 95% ethanol demonstrated the highest antioxidant activity. Although the aqueous extract produced the highest yield among all solvents, it exhibited the lowest antioxidant activity (Tables 1 and 5), supporting the view that extraction yield does not directly correspond to pharmacological activity. The different antioxidant activities of the extracts can be attributed to the solvents' varying capacities to dissolve antioxidant constituents such as phenolics and flavonoids, compounds widely acknowledged for their free radical scavenging potential (30). Ethanol, especially at high concentrations, has an intermediate polarity that enables it to extract both moderately polar and non-polar phytochemicals, thereby enhancing the antioxidant profile of the extract (27). In contrast, water, being highly polar, is less effective at extracting certain antioxidant compounds, particularly those that are weakly polar or tightly bound to plant cell wall structures. These findings align with previous studies reporting that hydroalcoholic solvents, particularly those containing 70%–95% ethanol, are more efficient at recovering antioxidant-rich phytochemicals than pure water (57). The antioxidant activity was correlated with the total phenolic and flavonoid contents, with the 95% ethanolic extract of *G. lanceifolia* leaves showing the richest phytochemical profile, including alkaloids, saponins, terpenoids, tannins, and steroids. These classes of compounds have been individually linked to antioxidant mechanisms. For instance, Macáková et al (58) demonstrated that certain alkaloids mitigate oxidative stress by inhibiting NADPH oxidase and activating the Nrf2 signaling pathway. Similarly, Wu et al (59) reported that saponins isolated from *Allium macrostemon* displayed potent antioxidant activity, with IC_{50} values comparable to Trolox. Additionally, terpenoids, which are fundamental structural units of saponins, are recognized for their ability to scavenge free radicals and regulate oxidative signaling pathways (60). Taken together, the superior antioxidant activity observed in the 95% ethanolic extract of *G. lanceifolia* emphasizes the importance of selecting an appropriate solvent system to optimize the extraction of bioactive compounds with potential therapeutic value.

Postprandial hyperglycemia, a critical contributor to the development of T2DM, can be effectively controlled by inhibiting the digestive enzymes α-amylase and α-glucosidase. α-Amylase initiates the breakdown of complex carbohydrates into smaller oligosaccharides, which α-glucosidase then converts into glucose in the small intestine. Blocking both enzymes slows down glucose release and absorption, thereby reducing spikes in blood sugar levels after meals (61). Consequently, plant extracts that inhibit these enzymes simultaneously are being increasingly studied as natural options for diabetes management (62). In this research, the inhibitory effects of *G. lanceifolia* leaf extracts on α-glucosidase and α-amylase were evaluated. The results in Table 6 demonstrate that solvent polarity plays a decisive role in modulating enzyme inhibition. Ethanol-rich extracts exhibited the strongest inhibitory activity, consistent with their higher phenolic and flavonoid contents, which are known contributors to carbohydrate-hydrolyzing enzyme inhibition (63). Importantly, these extracts also showed the highest antioxidant activities in both DPPH and ABTS

assays (Table 5), indicating a positive correlation between antioxidant potential and enzyme inhibition. The superior performance of 95% ethanol extracts highlights the efficiency of moderately polar solvents in concentrating antidiabetic and antioxidant phytochemicals. In contrast, the comparatively weak activity of the aqueous extract likely reflects its limited ability to solubilize non-polar or moderately polar bioactive compounds. The potent enzyme inhibition of the 95% ethanol extract is probably due to compounds such as dimethyl succinate, 2,5-diformylfuran, 2,4-di-tert-butylphenol, methylated benzenepropanoic acid derivatives, caryophyllenyl alcohol, hexadecanoic acid esters (methyl and ethyl), and bicyclosesquiphellandrene, as identified by GC-MS analysis (52-55). Many of these compounds are also recognized for their free radical-scavenging properties, which may further support enzyme modulation through oxidative stress mitigation. Although acarbose exhibited stronger inhibition, the ethanolic extracts of *G. lanceifolia* still showed notable dual activity against α-glucosidase and α-amylase, underscoring their potential as natural agents for glycemic control and functional food applications. To the best of our knowledge, this study is the first to demonstrate that ethanolic extracts of *G. lanceifolia* leaves can simultaneously inhibit both α-glucosidase and α-amylase. This finding advances the earlier report by Supasuteekul et al (18), which described only α-glucosidase inhibition using methanolic extracts, thereby emphasizing the distinctive role of ethanol as an extraction solvent. Ethanol not only enhances the recovery of phenolic and flavonoid compounds but also offers important benefits compared to methanol, including lower toxicity, greater safety for human use, and wider acceptance in functional food and nutraceutical development. Consequently, the present results point to the superior bioactivity and practical relevance of ethanol-based extracts. In line with evidence from related *Garcinia* species in the Clusiaceae family, such as *G. cowa* Roxb. (23), *G. gummi-gutta* (L.) Roxb. (64), and *G. indica* Choisy (kokum) (65), the observed enzyme inhibition may largely stem from phenolic and flavonoid compounds capable of modulating enzyme activity through active-site interaction or structural alteration (66). Moreover, their antioxidant potential could synergistically safeguard pancreatic β-cells and promote glycemic balance. Collectively, these findings support the ethnomedicinal use of *G. lanceifolia* and underscore the importance of further phytochemical isolation and in vivo validation of its bioactive constituents.

Conclusion

Overall, the 95% ethanolic extract of *G. lanceifolia* leaves demonstrated the richest phytochemical profile, with superior antioxidant activity and dual inhibition of α-glucosidase and α-amylase. Phytochemical screening, total phenolic and flavonoid quantification, antioxidant assays, and GC-MS profiling consistently indicated that 95% ethanol was the most effective solvent for recovering bioactive metabolites, maximizing both phytochemical diversity and pharmacological potential. The observed correlation between antioxidant capacity and enzyme inhibition supports the ethnomedicinal use of this species for glycemic regulation and highlights its promise as a natural therapeutic agent for diabetes management. These findings, reported here for the first time using ethanolic extracts, warrant further bioactivity-guided fractionation to isolate key active constituents, *in vivo* studies to validate their physiological efficacy, mechanistic investigations to elucidate compound-enzyme interactions, and exploration of advanced extraction techniques to enhance yield, efficiency, and sustainability.

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Conflict of interests

The authors declare that they have no conflict of interest.

Ethical considerations

The authors have paid close attention to ethical considerations concerning authorship, data collection, review, and analysis.

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