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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *ILLICIUM PARVIFOLIUM* LEAF ESSENTIAL OIL

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ABSTRACT. In this study, the chemical composition and the antibacterial and antidiabetic activities of the essential oil extracted from the leaves of *Illicium parvifolium* Merr. were evaluated. To the best of our knowledge, this study represents the first investigation of the phytochemical composition and biological activities of this species. The essential oil was obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry (GC–MS), resulting in the identification of 57 compounds, representing 98.95% of the total oil. The major classes of volatile constituents were monoterpene hydrocarbons (39.46%), oxygenated monoterpenes (35.60%), oxygenated sesquiterpenes (16.27%), and sesquiterpene hydrocarbons (7.53%). The dominant constituents were α-pinene (26.93%), eucalyptol (17.66%), linalool (11.25%), cubeban-11-ol (6.09%), and limonene (6.00%). The essential oil showed antibacterial activity against *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*, with minimum inhibitory concentration (MIC) values of 128-256 µg/mL. It also exhibited notable α-glucosidase inhibitory activity, with an IC_{50} value of 661.5 ± 17.6 µg/mL, whereas its inhibitory effect on α-amylase was weak, with an IC_{50} value greater than 1000 µg/mL. Overall, these findings indicate that *I. parvifolium* leaf essential oil is a promising source of natural antibacterial and antidiabetic agents.

KEY WORDS: *Illicium parvifolium* Merr., Essential oil, Antibacterial activity, α-Glucosidase inhibitory, Vietnam

INTRODUCTION

Genus *Illicium* (Schisandraceae) is widely distributed throughout Asia and North America and comprises more than 40 species, of which 10 species are found in Vietnam. Several *Illicium* species are economically and medicinally important owing to the presence of essential oils and structurally diverse secondary metabolites that are widely used in traditional medicine and the food flavoring industry [1, 2]. Previous phytochemical studies have revealed considerable variation in the chemical composition of *Illicium* essential oils. Phenylpropanoids such as (*E*)-anethole, myristicin, and safrole are often the dominant constituents of several species, including *I. henryi*, *I. difengpi*, *I. lanceolatum*, and *I. verum* [1-6], whereas monoterpenes, including 1,8-cineole, limonene, linalool, sabinene, and terpinen-4-ol have been reported as major components in *I. anisatum*, *I. griffithii*, and *I. pachyphyllum* [7-11]. Correspondingly, essential oils from *Illicium* species have been reported to possess anti-elastase, anti-inflammatory, antimicrobial, antinociceptive, antioxidant, cytotoxic, and insecticidal activities [1-6, 8, 11-15].

I. parvifolium Merr. is an evergreen tree native to southern China and Vietnam. The species is characterized by its small, leathery leaves, aromatic tissues, and distinctive star-shaped fruits, which are typical features of the genus. In Vietnam, it grows naturally in the humid tropical forests of the Hue and Da Nang regions. According to traditional Vietnamese medicine, *I. parvifolium* has been used as a tonic, a digestive stimulant, and an expectorant [16, 17]. Despite its traditional use and potential medicinal value, information on its phytochemical constituents and biological activities remains scarce. To the best of our knowledge, no previous study has investigated the

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chemical composition or biological activities of the essential oil from *I. parvifolium*. Therefore, the present study aimed to characterize the chemical composition of the leaf essential oil using GC-MS and to evaluate its antibacterial and α-glucosidase-inhibitory activities, thereby providing the first phytochemical and bioactivity data for this species.

EXPERIMENTAL

Plant materials

The fresh leaves (600 g) of *I. parvifolium* Merr. were collected from Phong Dien district, Hue city in February 2025 (16º31′14″N, 107º12′42″E), and identified by Dr. Le Tuan Anh, Viet Nam National Museum of Nature. A voucher specimen (MISR-25-05) has been deposited at the Mientrung Institute for Scientific Research, VNMN, VAST, Vietnam.

Figure 1. Leaf, flower, and fruit of *I. parvifolium* Merr.

Chemicals and reagents

Mueller-Hinton broth (MHB) and Mueller-Hinton agar (MHA) were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Dimethyl sulfoxide (DMSO, ≥ 99.9%, molecular biology grade), ciprofloxacin (≥ 98% purity), and C_7-C_{30} Saturated Alkanes were supplied by Sigma-Aldrich (St. Louis, MO, USA). Hexane (≥ 95% purity, GC/MS) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Porcine pancreatic α-amylase, α-glucosidase from *Saccharomyces cerevisiae*, starch azure, *p*-nitrophenyl-*α*-D-glucopyranoside (pNPG) (≥ 98% purity), acarbose (≥ 95% purity), and other analytical-grade reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Extraction of the essential oil

The extraction of essential oil from *I. parvifolium* leaves was performed according to the procedures described in previous reports [18, 19], with slight modifications. Fresh leaves were cut into 1-2 cm pieces and placed in a 2-L flask containing 200 g of plant material and 1,400 mL of distilled water (plant material-to-water ratio, 1:7, w/v). Hydrodistillation was carried out for 2 h using a modified all-glass Clevenger apparatus equipped with a 5 mL graduated essential oil receiver, in accordance with the Vietnamese Pharmacopeia V [20]. The distillation was considered complete when no further increase in essential oil yield was observed during two consecutive measurements performed at 20 min intervals. The obtained essential oil was dried

over anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture and subsequently stored at 4 ºC until further analysis. Essential oil yields were expressed as g/100 g of fresh plant material. Each distillation was conducted in triplicate to ensure reproducibility.

Analysis of the essential oil

The volatile constituents of the essential oil were analyzed using a TRACE 1310 GC coupled with an ITQ 900 ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a TG-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oil sample was diluted 1:100 (v/v) with *n*-hexane, and a 1 µL aliquot of the solution was injected. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector and transfer line temperatures were set at 260 ºC. The oven temperature was programmed as follows: 40 °C initially, increased to 240 ºC at a rate of 4 ºC /min, and held at 240 ºC for 5 min.

Mass spectra were acquired at an ionization energy of 70 eV over a scan range of m/z 45-500, with a scan rate of 1 scan/s. Volatile compounds were identified by comparing the obtained mass spectra with those in the NIST 14 (version 2.2) and Wiley 07 mass spectral libraries, as well as with published data [21, 22]. Experimental retention indices were calculated relative to a homologous series of *n*-alkanes (C_7-C_{30}) analyzed under identical chromatographic conditions. The relative abundance of each constituent was expressed as the percentage of its peak area relative to the total ion current (TIC).

Antibacterial assay

The antibacterial activity of the oil was evaluated against a panel of six reference bacterial strains: *Salmonella enterica* (ATCC 13076), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 14579), and *Staphylococcus aureus* (ATCC 25923). The minimum inhibitory concentrations (MICs) were determined using the broth microdilution method as previously described [23]. Briefly, the bacterial inoculum (100 µl at a concentration of 1×10^6 CFU/ml) was added to each well of 96-well microplates containing various concentrations of the oils (100 µl). Following incubation at 37 ºC for 24 h, bacterial growth was assessed by measuring the absorbance at 630 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). The MICs of the antibacterial oil were defined as the lowest concentration at which no bacterial growth was observed. Ciprofloxacin served as the positive control, and all experiments were performed in triplicate.

α-Amylase inhibitory assay

The α-amylase inhibitory activity of the essential oil was determined according to a previously established protocol [24]. The assay was carried out in 96-well microplates. Each well was loaded with 50 µL of the essential oil, 50 µL starch azure solution, and 50 µL of α-amylase (1.0 U/mL) in Tris-HCl buffer (pH 6.9). The reaction was incubated at 37 ºC for 10 min, and then was terminated by adding 50 µL of 50% acetic acid. Absorbance was recorded at 650 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). Acarbose was employed as the positive control. The percentage of α-amylase inhibitory activity was calculated using the formula:

$$\text{Inhibition (\%)} = (1 - A/A_c) \times 100,$$

Where A is the absorbance of the test sample, and A_c is the absorbance of the control. All experiments were conducted in triplicate, with each concentration tested in three replicate wells.

α-Glucosidase inhibitory assay

The α-glucosidase inhibitory activity of the essential oil was determined using a previously established protocol [24]. The assay was carried out in 96-well microplates. The reaction included 50 µL of the essential oil and 50 µL of α-glucosidase solution (0.5 U/mL) in phosphate buffer (0.1 M, pH 6.8). The reaction was incubated at 37 ºC for 10 min. Subsequently, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) was added as the substrate, and the reaction was further incubated at 37 ºC for 30 min. The reaction was then terminated by adding 50 µl of 0.2 M Na_2CO_3. Absorbance was recorded at 405 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). Acarbose was employed as the positive control. The percentage inhibition of α-glucosidase activity was calculated using the formula:

$$\text{Inhibition (\%)} = (1 - A/A_c) \times 100,$$

Where A is the absorbance of the test sample, and A_c is the absorbance of the control. All experiments were conducted in triplicate, with each concentration tested in three replicate wells.

Statistical analysis

All assays were conducted in triplicate, and the results are presented as mean ± standard deviation (SD). IC_{50} values were calculated by nonlinear regression analysis of concentration-response curves using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

The essential oil obtained from fresh leaves of *I. parvifolium* had a yield of 0.83 ± 0.07% (w/w, fresh weight basis). The oil appeared as a pale yellow liquid with a characteristic aromatic odor and was less dense than water. This yield is comparable to that reported for *I. lanceolatum* and is approximately three times higher than that of *I. anisatum* [8, 15]. The GC–MS analysis revealed that the oil contained 57 volatile compounds representing 98.95% of the oil content. The major chemical classes identified were monoterpene hydrocarbons (39.46%), oxygenated monoterpenes (35.60%), oxygenated sesquiterpenes (16.27%), sesquiterpene hydrocarbons (7.53%), and others (0.09%). The major constituents were α-pinene (26.93%), eucalyptol (17.66%), linalool (11.25%), cubeban-11-ol (6.09%), and limonene (6.00%).

Our findings demonstrate considerable variation in the chemical composition of essential oils among *Illicium* species compared with those previously reported. For example, the essential oil from leaves of *I. anisatum* is characterized by eucalyptol (21.8%), sabinene (5.3%), α-terpinenyl acetate (4.9%), and kaurene (4.5%), whereas the leaf essential oil of *I. lanceolatum* is predominantly composed of eucalyptol (42.42%), caryophyllene oxide (9.77%), and linalool (3.35%) [8, 15]. Moreover, the principal compounds in the essential oil from the fruit of *I. griffithii* were 4-methyl-6-(2-propenyl)-1,3-benzodioxole (22.64%), linalool (12.05%), caryophyllene (4.43%), and α-candinol (3.86%) [11]. In contrast, *I. pachyphyllum* fruit oil contained *trans-p*-mentha-1(7),8-dien-2-ol (24.56%), D-limonene (9.79%), caryophyllene oxide (9.32%), and cis-carveol (5.26%) [12]. Likewise, the stem bark essential oil of *I. difengpi* was dominated by safrole (23.61%), linalool (12.93%), germacrene D (5.35%), and 1,8-cineole (4.94%) [2], whereas the root essential oil of *I. henryi* primarily contained safrole (46.12%) and myristicin (20.39%) [1]. In addition, the fruit essential oil of *I. verum* consists almost entirely of *trans*-anethole (88.85-94.00%) [4, 5, 14, 25]. These findings highlight substantial interspecific variation in the chemical composition of essential oils from *Illicium* species, likely reflecting differences in genetic background, plant organ, and ecological or geographical conditions.

Table 1. Chemical composition of the leaf essential oil of *I. parvifolium.*

No	RT (min)	Compounds	RI[a]	RI[b] [21, 22]	Content (%)	Quality match (%)
1	8.23	*α*-Thujene	929	930	0.03	92
2	8.45	*α*-Pinene	935	939	26.93	98
3	8.92	Camphene	949	954*	0.54	84
4	9.11	2,4(10)-Thujadiene	955	960*	0.05	89
5	9.71	3,7,7-Trimethylcyclohepta-1,3,5-triene	973	970#	0.01	87
6	9.78	Sabinene	975	975	0.03	93
7	9.87	*β*-Pinene	978	979	0.60	96
8	10.39	*β*-Myrcene	994	990	0.50	97
9	10.83	Pseudolimonene	1006	1004	0.03	92
10	11.05	*δ*-3-Carene	1012	1011	0.24	91
11	11.59	*p*-Cymene	1027	1024	3.98	93
12	11.73	Limonene	1031	1029	6.00	97
13	11.81	Eucalyptol	1034	1031	17.66	92
14	12.07	*cis*-*β*-Ocimene	1041	1037	0.38	91
15	12.44	*trans*-*β*-Ocimene	1051	1050	0.12	95
16	12.81	*γ*-Terpinene	1061	1059	0.02	93
17	13.35	Linalool oxide B	1076	1072	0.13	89
18	13.93	Linalool oxide A	1092	1086*	0.15	88
19	14.24	*α*-Pinene oxide	1101	1099	0.09	95
20	14.38	Linalool	1105	1096*	11.25	98
21	*14.89*	*exo*-Fenchol	1119	1116	0.18	91
22	*16.81*	Borneol	1172	1169	0.41	89
23	17.16	Terpinen-4-ol	1182	1177*	0.95	92
24	17.68	Myrtenol	1197	1195	4.22	94
25	18.28	4-Methlyene-isophorone	1214	1217	0.07	88
26	19.88	*trans*-Ascaridol glycol	1260	1269	0.19	92
27	20.88	Bornyl acetate	1289	1285	0.06	90
28	21.37	4-Terpinenyl acetate	1303	1300	0.15	93
29	21.75	Myrtenyl acetate	1315	1326*	0.05	89
30	22.01	*p*-Mentha-1,4-dien-7-ol	1323	1327	0.04	87
31	23.02	*α*-Longipinene	1353	1352	0.07	90
32	23.90	*α*-Copaene	1380	1376	0.79	97
33	24.43	*β*-Elemene	1396	1396*	0.12	98
34	25.14	*cis*-*α*-Bergamotene	1419	1412*	0.72	93
35	25.33	*β*-Caryophyllene	1425	1419*	1.80	95
36	25.58	*β*-Gurjunene	1433	1433	0.04	94
37	25.79	Aromadendrene	1440	1441	0.25	91
38	25.94	Guaia-6,9-diene	1445	1444	0.59	92
39	26.42	*α*-Humulene	1460	1454	0.52	98
40	26.51	*allo*-Aromadendrene	1463	1460	0.21	93
41	26.62	9-*epi*-*β*-Caryophyllene	1466	1466	0.07	87
42	27.11	*γ*-Muurolene	1482	1479	0.13	89
43	27.44	*β*-Selinene	1493	1490	0.33	92
44	27.70	Viridiflorene	1501	1496	0.43	91
45	27.84	*α*-Muurolene	1506	1500*	0.21	94
46	28.07	*γ*-Cadinene	1514	1513	0.31	89
47	28.27	*β*-Cadinene	1520	1523	0.17	91
48	28.53	*δ*-Cadinene	1529	1523*	0.57	85
49	29.71	Germacrene B	1569	1561*	0.20	92
50	29.91	Caryophyllenyl alcohol	1576	1572	0.42	87

51	30.37	Cubeban-11-ol	1592	1595	6.09	90
52	30.66	Guaiol	1601	1600	1.13	92
53	30.95	Khusimone	1612	1604*	0.32	88
54	31.13	Humulene epoxide II	1618	1608*	0.51	94
55	32.08	*α*-Muurolol	1652	1646*	3.08	91
56	32.44	7-*epi*-*γ*-Eudesmol	1665	1663	4.72	89
57	32.81	Cadalene	1678	1676	0.09	86
Monoterpene hydrocarbon					39.46	
Oxygenated monoterpene					35.60	
Sesquiterpene hydrocarbon					7.53	
Oxygenated sesquiterpene					16.27	
Others					0.09	
Total					98.95	

Rt: retention time; RI[a]: retention indices calculated relative to *n*-alkanes (C_7-C_{30}) on a TG-5MS capillary column; RI[b]: literature retention indices [21]; RI[#] literature retention indices [22]; *Identified by comparison of mass spectra with the NIST 14 (version 2.2) and Wiley 07 mass spectral libraries, with confirmation by AMDIS 32 deconvolution software.

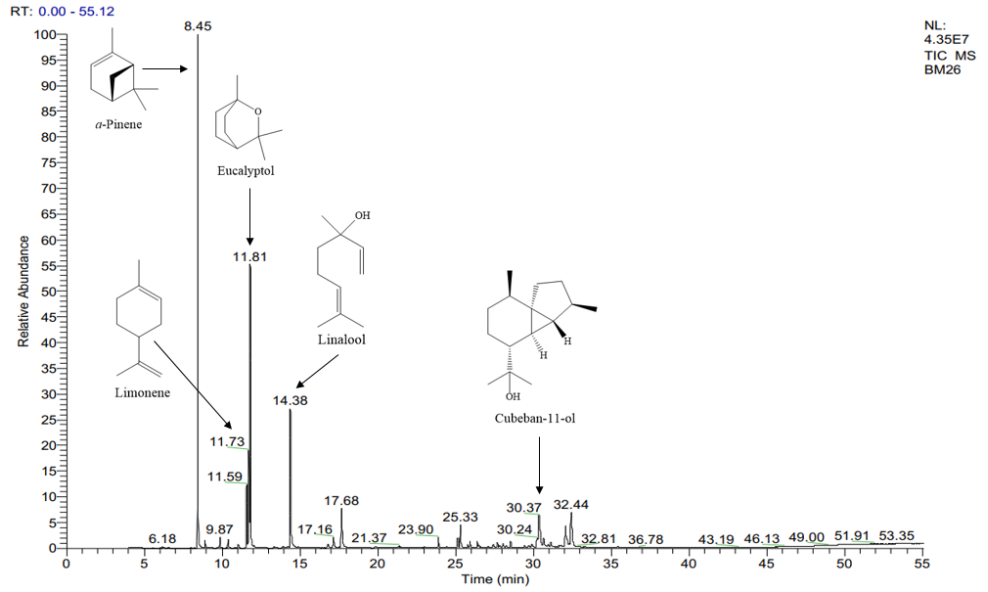

Figure 2. GC–MS spectrum of leaf essential oil of *I. parvifolium.*

Antibacterial assays showed that *I. parvifolium* essential oil inhibited the growth of all the tested bacterial strains, including *E. coli*, *S. enterica*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, and *B. cereus*, with MIC values ranging from 128 to 256 µg/mL (Table 2). The strongest antibacterial effect was observed against *P. aeruginosa* and *E. faecalis*, both with MIC values of 128 µg/mL. In contrast, *E. coli*, *S. enterica*, *S. aureus*, and *B. cereus* were less susceptible, exhibiting MIC values of 256 µg/mL. The results indicate that the essential oil possesses broad-spectrum antibacterial activity against both Gram-negative and Gram-positive bacteria. Although its antibacterial potency was lower than that of ciprofloxacin (MIC = 0.5-4 µg/mL), the observed MIC values suggest that the essential oil contains bioactive constituents capable of inhibiting bacterial growth. The major constituents of the essential oil, such as α-pinene, eucalyptol, linalool, and limonene, have each been reported to exhibit antimicrobial activity and are therefore likely to

contribute substantially to the antibacterial activity of the essential oil. For example, α-pinene exhibits antimicrobial activity, with MIC values of 5–10 µg/mL against a range of bacteria, including *B. subtilis*, *Enterobacter cloacae*, *E. coli*, *Micrococcus flavus*, *Proteus mirabilis*, *P. aeruginosa*, *Salmonella enteritidis*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, and *Staphylococcus aureus*. Eucalyptol and linalool also exhibit notable antibacterial effects, with MIC values of 4-9 µg/mL against several Gram-positive and Gram-negative species. Likewise, limonene, a major constituent of many citrus essential oils, demonstrates antimicrobial activity, with MIC values typically ranging from 7 to 15 µg/mL [26-28].

Table 2. Antibacterial activity of the *I. parvifolium* leaf essential oil.

Bacterial strains	MIC (µg/mL)	
	Essential oil	Ciprofloxacin
Escherichia coli ATCC25922	256	0.5
Salmonella enterica ATCC13076	256	1
Pseudomonas aeruginosa ATCC27853	128	2
Enterococcus faecalis ATCC29212	128	4
Staphylococcus aureus ATCC25923	256	1
Bacillus cereus ATCC14579	256	2

Furthermore, the essential oil of the *I. parvifolium* exhibited α-glucosidase inhibitory activity, with an IC_{50} value of 661.5 ± 17.6 µg/mL, compared with that of acarbose (IC_{50} = 117.7 ± 4.0 µg/mL). Conversely, the oil exhibited only weak inhibition of α-amylase, with an IC_{50} value exceeding 1,000 µg/mL (Table 3). The greater inhibitory activity against α-glucosidase than α-amylase may indicate a preferential interaction of certain essential oil constituents with α-glucosidase. The inhibitory activity of the essential oil may be attributed to major constituents, which have been reported to exhibit antidiabetic effects. Monoterpenes such as α-pinene and limonene have been shown to inhibit α-glucosidase *in vitro*. The major constituents of *Laurus nobilis* essential oil, including 1,8-cineole, 1-(*S*)-α-pinene, and *R*-(+)-limonene, have demonstrated α-glucosidase inhibition with IC_{50} values ranging from 1.118 to 1.748 µL/mL. Kinetic investigations further showed that 1,8-cineole competitively inhibited α-glucosidase, whereas 1-(*S*)-α-pinene and *R*-(+)-limonene acted as uncompetitive inhibitors [29]. In another investigation, α-pinene and limonene were reported to exhibit potent α-glucosidase inhibitory activity with IC_{50} of 95.62 ± 4.11 and 78.03 ± 2.31 µg/mL, respectively, compared to the reference α-glucosidase inhibitor, acarbose, with IC_{50} of 199.53 ± 3.26 µg/mL [30].

Table 3. Antidiabetic-related enzyme inhibitory activity of *I. parvifolium* leaf essential oil.

Sample	IC_{50} (mg/mL)	
	α-Amylase inhibition	α-Glucosidase inhibition
Essential oil	>1,000	661.5 ± 17.6
Acarbose	79.1 ± 2.6	117.7 ± 4.0

The overall bioactivity of the essential oil is likely not due to a single compound, but rather the result of additive or synergistic interactions among its constituents. Therefore, further studies, such as the isolation of individual constituents, bioactivity-guided fractionation, and molecular docking analyses, are required to identify the compounds primarily responsible for the observed biological activities.

CONCLUSIONS

This study reports the first analysis of the chemical composition and antibacterial and antidiabetic activities of the leaf essential oil of *I. parvifolium*. The oil obtained by hydrodistillation and analyzed by GC–MS contains 57 compounds (98.95%), dominated by monoterpene hydrocarbons (39.46%) and oxygenated monoterpenes (35.60%). The major constituents were α-pinene (26.93%), eucalyptol (17.66%), linalool (11.25%), cubeban-11-ol (6.09%), and limonene (6.00%). The oil exhibited antibacterial activity against six pathogenic bacteria, with MICs of 128–256 μg/mL, and showed α-glucosidase inhibition with an IC_{50} of 661.5 ± 17.6 μg/mL. These findings suggest *I. parvifolium* leaf essential oil as a potential source of natural antibacterial and antidiabetic agents.

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