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Comprehensive Analysis of *Hedera Helix* Leaves: Chemical Composition and its Antibacterial and Antidiabetic Potentials

Dinh Luu Nhat Quynh[2], Tran Chieu An[2], Doan Le Quynh Nhu[3], Tran Phan Huynh Nhu[2], Pham Hong Ngoc[2], and Phung Van Trung[1, 2*]

[1]Center for Research and Technology Transfer, Vietnam Academy of Science and Technology (VAST), Ha Noi City, 100000, Vietnam

[2]Institute of Applied Materials Science, Vietnam Academy of Science and Technology (VAST), Ho Chi Minh City 700000, Vietnam

[3]Hoang Hoa Tham High School, Ho Chi Minh City 700000, Vietnam
Corresponding Author Email: *trung_cnhh[at]yahoo.com;*
pvtrung[at]ict.vast vn

Abstract: ***This research aimed to study on chemical constituents and investigate bioactivities of Hedera helix, including antibacterial and antidiabetic. From the methanol extract of H. helix leaves collected in Da Lat province (Vietnam), four triterpene saponins were isolated and determined as α-hederin (1), hederacoside D (2), hederacoside B (3), and hederacoside C (4). Their chemical structures were elucidated by NMR. The fractions and isolated compounds of H. helix leaves showed low antibacterial activity against gram-positive (Staphylococcus aureus) and gram-negative (Pseudomonas aeruginosa) bacteria. In addition, these samples were found to have lowα-glucosidase inhibitory activity at tested concentration.***

Keywords: *Hedera helix*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, α-glucosidase, triterpene saponins.

1. Introduction

Hedera helix L. is a dioecious evergreen woody liana, one of the 15 species of the genus *Hedera* in the Araliaceae family. The leaves are coriaceous, measuring between 4 and 10 centimeters in length and width, with a cordate base. Palmately, the lamina has three to five lobes. The upper surface is dark green with lighter, radiating venation, whereas the lower surface is more grayish-green and the venation is clearly elevated. The flowering stems are composed of ovate-rhomboidal to lanceolate, 3 to 8-cm long leaves [1]. The flowers produced from summer to late autumn are tiny, greenish-yellow umbels 3-5 cm in diameter, and the fruits are small, black berries that ripen in winter. *H. helix*is native to Western, Central, and Southern Europe, and has been introduced to North America and Asia. It is a widespread favorite in many nations [2].

Hedera helix L. is not only noted as an attractive plant, but also has potentially dangerous effects such as bloody diarrhea, gastrointestinal irritation, contact dermatitis, and death caused by leaves and fresh fruits. However, the medical field treats begin warts due to the antioxidant, antillergic, and antispasmodic properties. Several authors have reported that dry extracts positively affect respiratory functions in children with chronic bronchial asthma, as wel as antibacterial, antihelmintic, leishmanicidal, and anti fungal properties [3, 4]. Early studies analyzed the antifungal [5] and antibacterial [6] activities of *H. helix* saponins.

Besides, multiple studies have indicated a complex chemical composition for ivy leaves: phenolic acids (caffeic acid, neochlorogenic acid, chlorogenic acid), flavonoids (quercetin, kaempferol, isoquecitrin), phytosterols (stigmasterol, sitosterol), polyacetylenes (falcarinol), hederagenin, oleanolic acid, and *Hedera* saponins [3, 7, 8]. Triterpene saponins are potent ingredients for herbal plant medical uses that has been reported as the primary components in the crude extract of ivy plants. α-Hederin, hederacoside B, hederacoside C, and hederacoside D were medicinally useful active constituents of ivy leaf [9].

Although extensive research has been conducted on the medicinal properties of *Hedera helix*, not many studies has examined the efficacy of the extract's ivy saponins against naturally occurring bacterial strains and its antidiabetes activity. Therefore, the present study aimed to isolate triterpene saponins, and evaluate the antidiabetic and antibacterial activities of *Hedera helix*.

2. Materials and Methods

2.1. Chemicals and reagents

Deionized water; HPLC grade acetonitrile, methanol, chloroform; and analytical grade formic acid (≥98%) were obtained from Scharlau (Barcelona, Spain). α-glucosidase (Lot 0000209485), p-Nitrophenyl-α-D-glucopyranoside (PNPG), acarbose, chloramphenicol, DMSO, disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$), and sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$) were purchased from Sigma-Aldrich Chemical Co. (Singapore).

2.2. Sample preparation

Hedera helix was collected from Da Lat province, Vietnam, and identified by botanist Tran Huu Dang, Southern Institute of Ecology (VAST). A voucher specimen (Code:

NaPro.33.1019) was deposited in the Center for Research, Testing Pharmaceutical Chemistry (IAMS-VAST). After collection, the leaves were cleaned, let air dry, and then chopped into pieces in preparation for extraction.

2.3. Extraction and isolation

Air-dried powder (4.9 kg) of ivy leaves was extracted with methanol for 24 hours. Extracted solution was filtered throughout filter paper, and the solvent was evaporated to dryness using on rotary evaporator system (BÜCHI R-300, Switzerland). After five times of extraction, crude extract was directly immersed and partitioned effectively with n-hexane and ethyl acetate to obtain yielding n-hexane (81.5 g), EtOAc (30.0g), residue (495.0g) extracts, respectively. The residue extract was subjected to fractionation over silica gel, eluting with MeOH in EtOAc (0-100%, step-wise) to give 3 fractions MA (15.0 g), MB (158.0 g), and MC (210.0 g). Fraction MC was separated by column chromatography (CC) over silica gel with a solvent mixture of $CHCl_3$-EtOAc-MeOH-H_2O (20: 50: 30: 0.5, v/v) to yield 5 fractions: MC1 (2.5 g), MC2 (49.8 g), MC3 (15.1 g), MC4 (4.3 g), and MC5 (5.0 g).

The fraction MC2 (25.0 g) was subjected to silica gel CC and eluted with $CHCl_3$-MeOH-H_2O (70: 30: 5, v/v) to obtain 5 sub-fractions: MC2a (0.1 g), MC2b (0.43 g), MC2c (2.46g), MC2d (4.39g), and MC2e (3.63g). Compounds 1 and 4 were obtained from fraction MC2b and MC2e, respectively, by recrystallization. Fraction MC2b and MC2c weresubjected to separation on HPLC (Hewlett Packard series 1100), with Phenomenex LUNA C18 (2), 250x10mm, 5µm (Phenomenex, USA), flow rate of 5mL/min, sample concentration 5%, inject volume 200µL, gradient of 65%-100% MeOH in water for 35 min, to yield compounds 2 and 3. Chemical structures of the isolated compounds (Figure 1) were determined by NMR.

2.4. Antibacterial activity

2.4.1. Preparation of test organism cultures

Bacterial strains: The antibacterial effectiveness of the extracts was evaluated using two bacterial strains *Staphylococcus aureus* ATCC 6358 and *Pseudomonas aeruginosa* ATCC 27853.

2.4.2. Inoculums preparation

The agar plates were incubated at 37°C for24 hours. A meat peptone broth (MPB) was used for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Then, determine the bacterial density by optical densitometry at 660 nm and adjust to the bacterial density in the range of 10^6-10^7 CFU/ml.

2.4.3. Qualitative antibacterial activity by agar well diffusion assay

Antibacterial activity of the extract was performed using the agar well diffusion method. Resistant bacterial strains were inoculated on meat peptone broth. Each culture was swabbed on the surface of sterile nutrient agar plate. In agar plate, ten wells were prepared with the help of sterilized cork borer of 8 mm diameter. The antibacterial ability of the substance is tested by aspirating 20-50 µl of sample solution of different concentrations into the wells on the agar plate covered with the test bacteria. Chloramphenicol (10mg/ml) and DMSO were used as positive and negative control, respectively. Every plate used according to the aforementioned procedure was performed in triplicate for statistical average. The active ingredient will diffuse into the agar, if it has antibacterial activity, it will inhibit the growth of bacteria and cause the appearance of an antibacterial ring [6].

2.5. Antidiabeticactivity

α-Glucosidase activity was assessed by monitoring the formation of p-nitrophenol (PNP) from p-nitrophenyl-α-D-glucopyranoside (PNPG). Acarbose was used as positive control. Sodium phosphate buffer (0.1 M, pH 6.8) was used as reaction buffer. The samples were dissolved in DMSO 10% at a concentration of 1000 ppm. In each microplate well, 20 µL of test sample (or 20 µL of solvent for the negative controls), 20 µL of α-glucosidase (0.33U/mL) in water, and 140 µL of buffer were incubated at 37°C for 20 min. Then, 20 µL of PNPG substrate (2.5 mM) in water (or 20 µL of water for the blanks) was added to each well to initiate the reaction. After incubating at 37°C for 30 min, athermostatically controlled PowerWave HT microplate spectrophotometer (BioTek, USA) was used to measure the absorbance at 405 nm.

The percent inhibition was calculated as follows:

α-glucosidase inhibitory activity (%) = ((A0-A1))/A0× *100*%

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The IC_{50} value was defined as the sample concentration required to inhibit 50% α-glucosidase activity [10].

2.6. Data analysis of bioactivities

Data were processed using Rstudio (version 1.4.1717) software. The results were compared by one-way ANOVA and Tukey's HSD post hoc test. A difference was considered statistically significant if $p \leq 0.05$.

3. Results and Discussion

3.1. Structural determination of isolated compounds

3.1.1. Compound 1

^{1}H-NMR (DMSO-d_6, 500 MHz) spectrum of **compound 1** digital compound for 3 signals of CH_3 group at δ_H 0.57 (s); 1.07 (s); 1.08 (s), 3 CH_3 groups give 1 δ_H 0.87 (s) signal, 1 H-23 oxymethylene group at δ_H 3.12 and 3.28, 1 oxymethine H-3 group at δ_H 3.49 (1H, m). The ^{1}H-NMR spectrum also shows the presence of an olefin proton signal at δ_H 5.16 that characterizes H-12 in the structural framework of the 5-ring triterpene.

The **^{13}C-NMR** and **DEPT** spectroscopy of **compound 1** exhibit resonant signals of 41 carbons, 30 of which are consistent with published data for the structure of the triterpenoid framework as hederagenin including the signal of carbon olefins C-12 at δ_C 121.5 corresponding to carbon

methine and tetracarbon C-13 at δ_C 143.8 also confirms the existence of double bonds in the ring in the position C-12 position, and also C-28 signaling at δ_C 178.6 of the carboxyl group. In addition, the remaining 11 carbon signals showed the presence of 2 sugar units with resonant signals of 2 anomeric carbons C-1′ at δ_C 102.9 and C-1″ at δ_C 99.9.

The protons in the ring of sugar units are assigned precisely by means of **COSY, HSQC** and **HMBC** spectroscopy starting from the anomeric protons that have been identified. The chemical displacement of C-3 at δ_C 79.3 on the ^{13}C-NMR spectrum suggests that sugar circuits are attached to this position. Binding to sugar units was confirmed by HMBC interactions between the anomeric proton H-1' of arabinose at δ_H 4.63 with C-3 at δ_C 79.3, H-1" of rhamnose at δ_H 5.06 and C-2' of arabinose at δ_C 74.2.

HMBC spectroscopy shows that the correlation of protons H-24 at δ_H 0.57 and H-3 at δ_H 3.49 co-interacting with C-23 at δ_C 62.5 helps determine the OH group attached to the C-23 position. The correlation of two protons H-5′ at δ_H 3.44 and δ_H 3.66 with C-1′ at δ_C 102.9 helped determine that the binding sugar root at C-3 is the arabinose sugar. Hence, **compound 1** was determined as α-hederin.

3.1.2. Compound 2

^{1}H-NMR (DMSO-d_6, 600 MHz) spectrum of **compound 2**gives 3 signals of the methyl group at δ_H 0.59 (s); 0.68 (s); 1.08 (s), 3 methyl groups for 1 δ_H 0.88 (s) signal, 1 oxymethylene H-23 group at δ_H 3.18 and 3.40, 1 oxymethine H-3 group at δ_H 3.48 (1H, m). The ^{1}H-NMR spectroscopy also shows the presence of an olefin proton signal at δ_H 5.17 that characterizes H-12 in the structural framework of the five-ring triterpene.

The **^{13}C-NMR** and **HSQC** spectrum of **compound 2** exhibit synergistic signals of 53 carbons, 30 of which are consistent with published data for the structure of the triterpenoid framework as hederagenin including the signal of carbon olefin C-12 at δ_C 121.6 corresponding to carbon methine and tetracarbon C-13 at δ_C 143.4 also confirms the existence of double bonds in the ring in position C-12 position, and also the C-28 signal at δ_C 175.2 of the carboxyl group. In addition, the remaining 23 carbon signals showed the presence of 4 sugar units with resonant signals of 4 anomeric carbons C-1′ at δ_C 104.7, C-1″ at δ_C 93.9, C-1″ at δ_C 93.9, C-1‴ at δ_C 102.5, C-1⁗ at δ_C 100.5.

The **^{1}H-NMR and HSQC** spectroscopy also confirmed that four corresponding anomeric protons interact with four anomeric carbons: H-1′ at δ_H 4.19 (1H, d, 6.6), H-1″ at δ_H 5.22 (1H, d, 7.8), H-1‴ at δ_H 4.27 (1H, d, 7.8), H-1⁗ at δ_H 4.70 (1H, d, 3.6). The ^{1}H-NMR signal spectroscopy of a methyl group at δH 1.08 indicates the existence of a deoxy sugar in the structure of **compound 2**.

The protons in the ring of sugar units are assigned precisely by means of **COSY, HSQC and HMBC** spectroscopy starting from the anomeric protons that have been identified. The chemical displacements of C-3 at δ_C 79.8 and C-28 at δ_C 175.2 on the 13C-NMR spectrum suggest that sugar circuits are attached to these sites. Binding to sugar units was confirmed by HMBC interactions between the anomeric proton H-1' of arabinose δ_H 4.19 (1H, d, 6.6) with C-3 at δ_C 79.8, H-1" of glucopyranose δ_H 5.22 (1H, d, 7.8) with C-28 at δ_C 175.2, H-1‴ of glucopyranose δ_H 4.27 (1H, d, 7.8) with C-6″ at δ_C 67.5, and H-1⁗ of rhamnose δ_H 4.70 (1H, d, 3.6) with C-4‴ at δ_C 76.8. Thus, **compound 2** was determined as hederacoside D.

3.1.3. Compound 3

The **^{1}H-NMR** (DMSO-d_6, 500 MHz) spectrum of **compound 3** gives 4 signals of the methyl group at δ_H 0.68 (s); 0.75 (s); 0.93 (s); 1.08 (s), 3 methyl groups give 1 δ_H 0.86 (s) signal, 1 oxymethine H-3 group at δ_H 3.00 (1H, m). The ^{1}H-NMR spectroscopy also shows the presence of an olefin proton signal at δ_H 5.17 that characterizes H-12 in the structural framework of the five-ring triterpene.

The **^{13}C-NMR and DEPT** spectroscopy of **compound 3**show synergistic signals of 59 carbons, 30 of which are consistent with published data for the structure of the triterpenoid framework as oleanoic acid including signals of olefin C-12 carbon at δ_C 121.6 corresponding to methine carbon and C-13 tetracarbon at δ_C 143.4 also confirming the existence of double bonds in rings at the C-12 position, and also the C-28 signal at δ_C 175.2 of the carboxyl group. In addition, the remaining 29 carbon signals showed the presence of 5 sugar units with resonant signals of 5 anomeric carbons C-1′ at δ_C 103.7, C-1″ at δ_C 99.9, C-1‴ at δ_C 93.9, C-1⁗ at δ_C 102.5, C-1‴ at δ_C 100.5; 19 oxymethine group signals, 3 oxymethylene group C-5′ signals at δ_C 63.7, C-6‴ at δ_C 67.8, C-6⁗ at δ_C 59.9 and 2 methyl group signals C-6″ at δ_C 17.7 and C-6⁗ at δ_C 17.7.

The **^{1}H-NMR and HSQC** spectroscopy also confirmed that there are five corresponding anomeric protons interacting with five anomeric carbons: H-1′ at δ_H 4.30 (1H, d, 5.4), H-1″ at δ_H 5.03 (1H, m), H-1‴ at δ_H 5.22 (1H, d, 7.8), H-1⁗ at δ_H 4.27 (1H, d, 7.8) and H-1⁗ at δ_H 4.68 (1H, brs). The ^{1}H-NMR signaling spectroscopy of 2 methyl groups at δ_H 1.08 indicates the existence of 2 deoxy pathways in the structure of **compound 3.**

The protons in the ring of sugar units are assigned precisely by means of **COSY, HSQC and HMBC** spectroscopy starting from the anomeric protons that have been identified. The chemical shifts of C-3 at δ_C 87.7 and C-28 at δ_C 175.2 on the ^{13}C-NMR spectrum suggest that sugar circuits are attached to these sites. Binding to sugar units was confirmed by HMBC interactions between the anomeric proton H-1' of arabinose δ_H 4.30 with C-3 at δ_C 87.7, H-1" of rhamnose δ_H 5.03 with C-2' of arabinose at δ_C 74.4, anomeric proton H-1‴ of glucopyranose δ_H 5.22 (1H, d, 7.8) and C-28 at δ_C 175.2, anomeric proton H-1⁗ of glucopyranose δ_H 4.27 (1H, d, 7.8) with C-6‴ at δ_C 67.8, anomeric proton H-1⁗ of rhamnose δ_H 4.68 (1H, br. s) with C-4⁗ at δ_C 75.1. As a result, **compound 3**was determined as hederacoside B.

3.1.4. Compound4

^{1}H-NMR (DMSO-d_6, 500 MHz) spectrum of **compound 4** gives 3 signals of the methyl group at δ_H 0.57 (s); 0.68 (s); 1.06 (s), 3 methyl groups give 1 δ_H 0.87 (s) signal, 1 oxymethylene H-23 group at δ_H 3.14 and 3.27, 1 oxymethine H-3 group at δ_H 3.35 (1H, m). The ^{1}H-NMR spectroscopy also shows the presence of an olefin proton signal at δ_H 5.18

that characterizes H-12 in the structural framework of the 5-ring triterpene.

The **^{13}C-NMR** and **DEPT** spectroscopy of**compound 4** exhibit resonant signals of 59 carbons, 30 of which are consistent with published data for the structure of the triterpenoid framework as hederagenin including the signal of carbon olefins C-12 at δ_C 121.8 corresponding to carbon methine and carbon tetralevel C-13 at δ_C 143.5 also confirms the existence of double bonds in the ring in position C-12 position, and also C-28 signaling at δ_C 175.4 of the carboxyl group. In addition, the remaining 29 carbon signals showed the presence of 5 sugar units with resonant signals of 5 anomeric carbons C-1′ at δ_C 102.9, C-1″ at δ_C 99.9, C-1‴ at δ_C 94.0, C-1⁗ at δ_C 102.5, C-1⁗ at δ_C 100.6.

The **^{1}H-NMR** and **HSQC** spectroscopy also confirmed that there are five corresponding anomeric protons interacting with five anomeric carbons: H-1′ at δ_H 4.33 (1H, d, 7.2), H-1″ at δ_H 5.06 (1H, brs), H-1‴ at δ_H 5.21 (1H, d, 9.6), H-1⁗ at δ_H 4.27 (1H, d, 9.0) and H-1⁗ at δ_H 4.87 (1H, d, 5.4). The ^{1}H-NMR signaling spectroscopy of two methyl groups at δ_H 1.1 indicates the existence of two deoxy pathways in the structure of **compound 4.**

The protons in the ring of sugar units are assigned precisely by means of **COSY, HSQC** and **HMBC** spectroscopy starting from the anomeric protons that have been identified. The chemical displacements of C-3 at C-3 atδ_C 79.3 and C-28 at δ_C 175.4 on the ^{13}C-NMR spectrum suggest that sugar circuits are attached to these sites. Binding to sugar units is confirmed by HMBC interactions between anomeric protons (H-1') of arabinose δ_H 4.63 with C-3 at δ_C 79.3, H-1" of rhamnose at δ_H 5.06 with C-2' of arabinose δ_C 74.2, anomeric proton H-1⁗ of glucopyranose δ_H 5.21 (1H, d, 9.6) and C-28 at δ_C 175.4, anomeric proton H-1⁗ of glucopyranose at δ_H 4.27 (1H, d, 9.0) with C-6‴ at δ_C 67.6, anomeric proton H-1⁗ of rhamnose at δ_H 4.87 (1H, d, 5.4) with C-4⁗ at δ_C 78.5.

HMBC spectroscopy shows that the correlation of protons H-24 at δ_H 0.57 and H-3 at δ_H 3.35 co-interacting with C-23 at δ_C62.5 helps determine the OH group attached to the C-23 position. Therefore, **compound 4** was determined as hederacoside C.

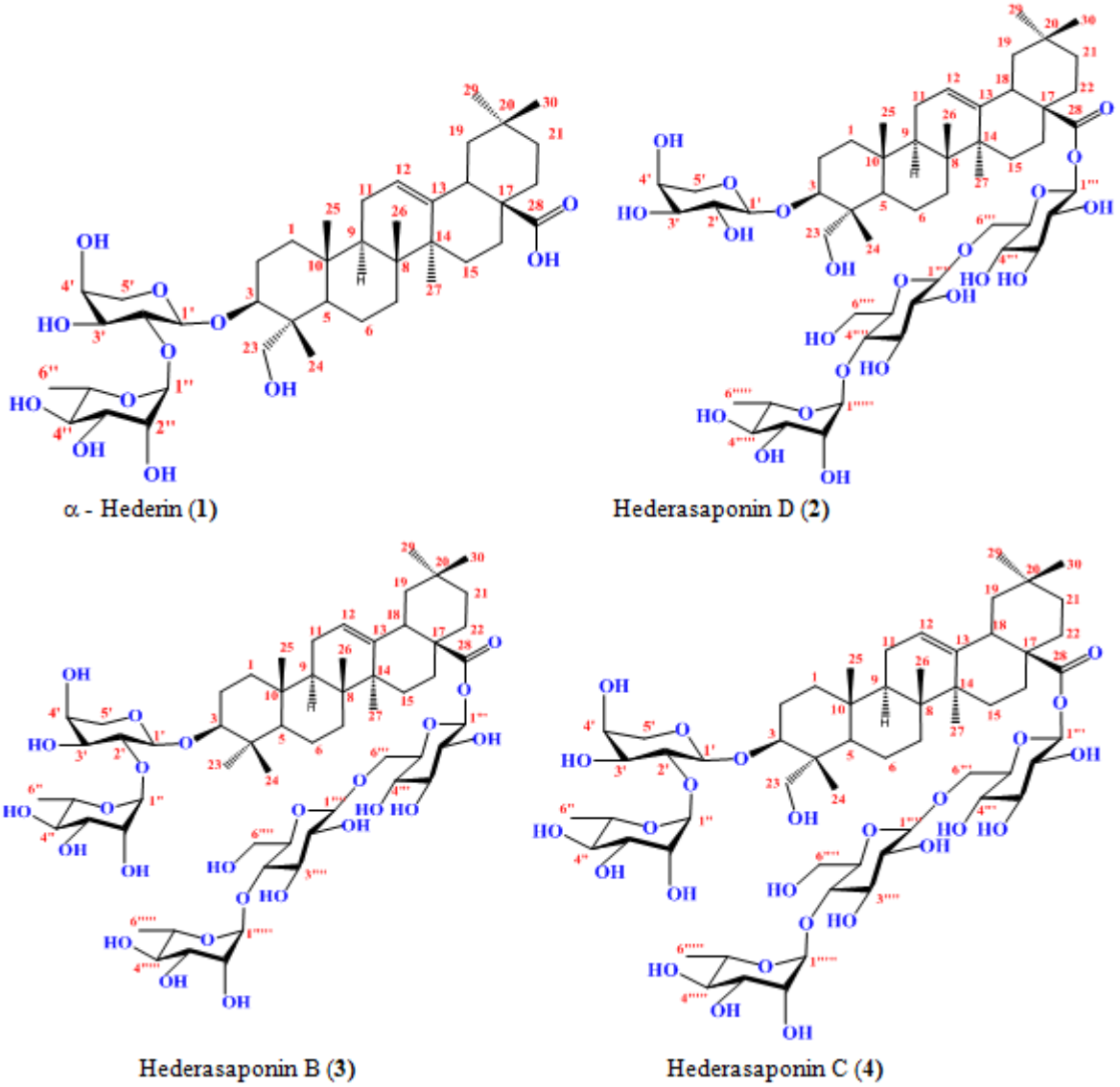

Figure 1: Chemical structures of isolated compounds from *H. helix*.

3.2. Antibacterial activity

The antimicrobial activity of the extracts from *Hedera helix* leaves was investigated by the agar well diffusion method to determine the antibacterial ability of the extracts against some bacterial strains associated with respiratory diseases. Mean zones of inhibition were expressed in mm ± standard error of mean (Table 1).

From the results in Table 1, hederacoside B, C, and D had lower antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* than the fractions, indicating that the bioactive compounds were not these saponins. The result was similar when evaluating the activity of α-hederin against *Staphylococcus aureus*, but when investigated on *Pseudomonas aeruginosa*, α-hederin showed slightly higher activity than the other three saponins and was comparable to the fraction used to isolate it (MC2b). Thus, it can be inferred that α-hederin is the active compound against *Pseudomonas aeruginosa* isolated from MC2b. However, this result is still very low.

Antibiotic resistance remains a concern in a number of developing and industrialized countries, posing a significant hazard to the global health sector. Due to the inefficacy of presently available antimicrobial for the treatment of infectious diseases, numerous researchers have turned to natural products as potential sources of novel bioactive compounds [11, 12]. In this investigation, the antibacterial activity of the samples was not against *Pseudomonas aeuroginosa* and *Staphylococcus aureus*. Therefore, the pharmacological activity of *Hedera helix* could not be confirmed. These findings are inconsistent with previous studies. It is possible that the growing conditions affect the chemical composition, which causes the antibacterial performance of the samples to change. However, it is also possible that the active ingredient with antibacterial activity in *Hedera helix* is different from the four isolated phytochemicals. To ascertain their effectiveness in inhibiting the development of the bacteria, additional research is required.

Table 1: Antibacterial activity of the samples from *Hedera helix*

		Zone of Inhibition (zone diameter-mm)	
Sample	(mg/well)	*Staphylococcus Aureus* ATCC 6538	*Pseudomonas Aeruginosa* ATCC 27853
MC2	1.52	8.6 ± 0.1^{a}	$12.0 \pm 0.3^{a, c}$
α-hederin	1.50	$8.5 \pm 0.2^{a, b}$	12.2 ± 0.3^{a}
Hederacoside D	1.52	8.5 ± 0.3^{a}	9.2 ± 0.2^{b}
Hederacoside B	1.51	8.5 ± 0.2^{a}	9.1 ± 0.3^{b}
Hederacoside C	1.52	8.2 ± 0.2^{a}	9.0 ± 0.2^{b}
MC2a	1.51	9.5 ± 0.3^{a}	$12.0 \pm 0.3^{a, d}$
MC2b	1.50	11.0 ± 0.4^{c}	12.3 ± 0.4^{a}
MC2c	1.53	14.0 ± 0.4	$12.0 \pm 0.4^{a, e}$
MC2d	1.50	9.2 ± 0.5^{b}	$10.0 \pm 0.3^{b, f}$
MC2e	1.51	12.0 ± 0.3^{c}	$11.0 \pm 0.5^{c, d, e, f}$
Chloramphenicol		22.0 ± 0.5	21.0 ± 0.3

(Mean values followed by the same uppercase letter in each group are not significantly different from each other at p≤0.05 according to Tukey's HSD test).

3.3. α-glucosidase inhibitory activity

To evaluate antidiabetic potential of *Hedera helix* species, α-glucosidase inhibitory activity of the fractions and isolated compounds from this species was performed. The results were shown in Table 2. As a result, fraction MC2 of *H. helix*extract showed promising α-glucosidase inhibition (45.23%). Among the 4 isolated compounds, hederacoside C exhibited the strongest α-glucosidase inhibition activity (35.48%). The other three isolated saponins including hederasaponin B, hederasaponin D, and α-hederin showed low inhibitory activity (16.99, 14.45, and 11.34%, respectively). The results indicated that triterpene saponins in *H. helix* species were not the active phytochemicals with inhibitory activity of α-glucosidase enzyme from this medicinal species. In order to identify potential antidiabetic compounds from *H. helix* species, further studies are needed to isolate other compounds and test their α-glucosidase enzyme inhibitory activity.

Table 2: α-glucosidase inhibitory activity of *H. helix* fractions and isolated compounds at concentration of 100ppm

Sample	Inhibitory activity (%)
MC2	45.23 ± 0.18
α-hederin	11.34 ± 0.03
Hederacoside D	14.45 ± 0.07
Hederacoside B	16.99 ± 0.08^{a}
Hederacoside C	35.48 ± 0.12
MC2a	13.84 ± 0.03
MC2b	$17.09 \pm 0.05^{a, b}$
MC2c	30.73 ± 0.11
MC2d	16.11 ± 0.04
MC2e	17.42 ± 0.04^{b}
Acarbose	85.39 ± 0.23

(Mean values followed by the same uppercase letter are not significantly different from each other at p≤0.05 according to Tukey's HSD test).

4. Conclusion

This study was initiated with the aim of evaluating the antibacterial and inhibitory activity of α-glucosidase enzyme from *H. helix* species. In an effort to purify possible pure saponins compounds in *H. helix* species, four tritepene saponins including hederacoside B, hederacoside C, hederacoside D, and α-hederin were successfully isolated. The results of antibacterial testing showed that fractions and active ingredients from *H. helix* species had low effectiveness against *Pseudomonas aeuroginosa* and *Staphylococcus aureus* by agar well diffusion method. In addition, although the MC2 fraction had potential α-glucosidase inhibitory activity, the subfractions and four isolated compounds did not really show potential in the treatment of diabetes. In order to identify potential antidiabetic compounds from *H. helix* species, further studies are needed to isolate other compounds and test their α-glucosidase enzyme inhibitory activity.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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