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Optimised extraction conditions for maximising saponin yield and antioxidant properties of *Hedera helix* L.

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Hedera helix L. (*H. helix*) has been known as a potential medicinal plant in folk medicine for the treatment of different chronic diseases. Recently, several phytochemical compounds were extracted from the *H. helix*, especially the saponin group. This research aimed to optimise extraction conditions to obtain the highest saponin yield from the *H. helix*. Total saponin content (TSC), saponin extraction efficiency (SEE), total phenolic content (TPC), total flavonoid content (TFC), DPPH/ABTS radical scavenging capacity (DRSC/ARSC) and ferric reducing antioxidant power (FRAP) in the extract were determined. The results show that the highest TSC (52.57 mg escin equivalents (EE)/g dry weight (DW) and SEE (72.21%) was achieved from the *H. helix*. using conventional extraction (CE) with 80% methanol (v/v), 1:100 (w/v) sample to solvent ratio, 50°C extraction temperature, and 30 min extraction time. In this condition, TPC and TFC (4.35 mg gallic acid equivalents (GAE)/g DW and 0.22 mg catechin equivalents (CE)/g DW, respectively) and DRSC, ARSC and FRAP (6.41, 15.35, and 8.26 mg trolox equivalents (TE)/g DW, respectively) were also identified. This study suggests the optimised extraction conditions to achieve the maximum TSC from the *H. helix*, which offers many benefits for functional food manufacturers.

Keywords: Antioxidant activity, Extraction conditions, *Hedera helix* L., Optimisation, Phytochemical compounds

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Introduction

The *Hedera helix* L., or ivy, is a member of the genus *Hedera* of the family *Araliaceae*. It is an evergreen climbing plant that is primarily found in western Asia and Europe. This plant grows in Vietnam's cool climatic regions, including Da Lat, Moc Chau, and Sapa[1]. Due to its inherent qualities, the *H. helix* is also grown as an ornamental plant in many places. Furthermore, the *H. helix* is regarded as a medicinal herb. Traditional medicine utilised all of this plant's organs to treat a variety of respiratory tract problems as well as inflammatory, bacterial, bodily detoxification, and antioxidant conditions[2]. The *H. helix* leaves were decocted in Turkey to treat diabetes[3], whereas Chinese physicians employed this plant to treat blood-vomiting, hepatitis, arthritis, and headaches[4]. The potential bioactivities of *H. helix* have recently been shown, including the treatment of respiratory tract issues[5,6], anti-inflammatory, antimicrobial, antioxidant[7], anticancer[8], antifungal[9], anti-arthritic[7], antiviral[10], and antidiabetic[11]. The saponin group, which is primarily in charge of the plant's functions, is particularly abundant in the *H. helix*. Moreover, Hederacoside C, a main active saponin isolated from *H. helix*, has been reported with its pharmacological properties in the treatment of health problems, including osteoarthritis[12] and acute lung inflammation[13]. Recently, although there is a lot of research on the identification of bioactive compounds in the *H. helix* and their pharmacological properties, the study of optimising extraction conditions for the constituents of *H. helix* has been limited.

In the investigation and utilisation of medicinal plants, extraction is an essential process in separating desirable components from plant materials. Factors such as solvents, extraction methods, temperature,

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extraction duration, and the sample to solvent ratio[14] significantly impact the efficiency of extracting phytochemical compounds, their bioactivities, and their effects on human health and the environment. Additionally, production costs, including extraction time, solvent volume, solvent recoverability, and energy consumption, should also be considered[15]. The choice of solvent greatly impacts the extraction yield of phytochemical compounds in plants. No single solvent can efficiently extract all phytochemicals from plant materials, as bioactive compounds differ in polarity and require solvents with matching polarities for optimal extraction[16,17]. Therefore, the selection of a solvent depends on the chemical characteristics of the desirable compounds. Non-polar solvents have a stronger affinity for non-polar molecules, while polar solvents are better at attracting polar ones. The polarity of a solvent determines its ability to dissolve and extract specific types of compounds. Typically, a combination of polar and non-polar solvents is used to enhance extraction efficiency[18,19]. The effective extraction of phytochemical components from herbs depends critically on the solids-to-liquid ratio. By improving mass transfer, using more solvent improves the collection of phytochemicals from the plant matrix[20]. However, it is important to select an acceptable sample to solvent ratio, taking into account its effect on manufacturing costs and energy usage. The temperature and extraction duration are crucial factors influencing the yield of extraction. The extraction medium's surface tension and viscosity decrease as temperature rises, which also accelerates mass transfer, increases molecular collision, and improves solubility[21]. Similarly, the extraction efficiency generally improves with longer extraction times. However, there is a saturation point where the extraction reaches equilibrium between the plant's internal substances and those already dissolved in the solvent. At this point, further extraction of phytochemical components ceases[21]. Furthermore, prolonged exposure to high temperatures might cause some phytochemical compounds to become unstable. Finally, extraction techniques play a significant role in collecting phytochemical constituents from plants. There are two main types: conventional extraction and advanced extraction. The most common conventional method is maceration, where solubility and diffusivity are key factors influencing its efficiency. Solubility refers to the dissolution of chemicals based on polarity, while diffusivity relates to the rate of mass transfer of phytochemical compounds into the solvent due to concentration gradients at the solid-liquid interface. Recently, advanced techniques have been introduced with their benefits, such as minimal hazardous chemical use, energy efficiency, reduced consumption of renewable feedstock, and pollution prevention. Ultrasound-assisted extraction (UAE) utilises acoustic cavitation in the solvent, which leads to various phenomena like fragmentation, erosion, greater shear force, enhanced mass transfer, and solubility of bioactive substances by damaging the cellular structure of plants[22]. In addition, microwave-assisted extraction (MAE) is another advanced technique that utilises electromagnetic waves. It generates heat through the interaction of microwaves with solvents and polar compounds in the plant material, leading to superheating and pressure buildup within the plant cells. This weakens the cell walls, facilitating the diffusion of phytochemical components into the solvent[23]. Therefore, it is crucial to identify the optimal extraction conditions for phytoconstituents from the medicinal plant *H. helix*

With this in mind, this research aimed to focus on the optimisation of extraction conditions in order to obtain the highest saponin content from the *H. helix*, which must be satisfied for the requirements of the environment, production cost and health benefits for sustainable development.

Material and Methods

Plant material

The *H. helix* was obtained in its entire parts from the Doly farm, Xuan Tho commune, Da Lat city, Lam Dong province, Vietnam (latitude 11.930655°N, longitude 108.515452°E) on 6 April 2023 and was identified by the National Institute of Medicinal Materials, Ministry of Health, Vietnam, according to Circular 38/2021/TT-BYT. To reduce oxidation, the fresh plants were then kept inside sealed plastic bags covered with ice. The collected samples were promptly transported to the Laboratory of the Research, Development, and Teaching Group on Functional Foods (Nha Trang University, Vietnam), where they were thoroughly washed with deionised water to remove impurities and degraded tissues. The samples were completely drained and divided into smaller pieces before being placed in aluminium foil zip-lock bags and stored at -20°C.

Analytical chemicals

All chemicals used in this study were of analytical grade. Chloroform, methanol, Folin–Ciocalteu reagent, sodium carbonate, sodium hydroxide, aluminum chloride, vanillin, sulfuric acid, Bromocresol Green, citric acid, disodium phosphate, hydrochloric acid, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulfate, DPPH (2,2-diphenyl-1-picryl-hydrazyl), copper(II) chloride, ammonium acetate, neocuproine, sodium acetate, TPTZ (2,4,6-tripyridyl-s-triazine), iron(III) chloride, gallic acid, catechin, escin, atropine, and Trolox were obtained from Sigma-Aldrich (Missouri, USA) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Preparation of the dried sample

The samples were thawed at room temperature and subsequently dried using hot air at 100°C for 4 h[24]. After drying, the samples were ground and stored in aluminium foil zip-lock bags at 4°C. The residual moisture content of the *H. helix* sample, as determined using the AOAC official methods (AOAC, 1998), was found to be 4.04% after heating in a hot-air oven at 105°C for 5 hours.

Preparation of *H. helix* extracts

This study employed a single-factor experimental design. Initially, the *H. helix* was extracted using conventional extraction (CE) method under fixed conditions: sample to solvent ratio (1:100 (w/v)), 30-minute rest at room temperature, extraction duration (30 min), and extraction temperature (50°C). After each investigation, these fixed conditions were replaced with correspondingly optimised conditions that were selected based on the study's objectives. The extraction procedures were implemented as follows:

To evaluate the factors influencing the extraction efficiency, various conditions were examined. Seven different solvents were tested, including water, pure methanol (MeOH), pure ethanol (EtOH), pure acetone (Ace), and their 50% aqueous solutions. Three extraction methods comprising CE, UAE, and MAE were employed. In which, UAE was carried out at 1500 W and 50°C, and MAE was performed at 270 W with 5 s/min irradiation cycles. The effects of solvent concentration were investigated by extracting *H. helix* using MeOH at concentrations of 60, 70, 80, 90, and 100%. Extraction temperature and duration were also optimized, with temperature levels of 40, 50, 60, and 70°C, and extraction times of 20, 30, 40, 50, and 60 minutes. Additionally, various sample-to-solvent ratios (1:20, 1:40, 1:60, 1:80, 1:100, 1:120, and 1:140, w/v) were evaluated. Upon completion of each extraction, the mixtures were rapidly cooled to room temperature using an ice-water bath, filtered through Whatman No. 1 filter paper, and adjusted to the desired volume with the corresponding solvent. The final extracts were stored at 4°C for subsequent analysis.

Total saponin content (TSC)

The TSC analysis of *H. helix* extracts follows the depicted method with some modifications[25]. In brief, 0.25 mL of the extract was combined with 0.25 mL of an 8% vanillin solution (w/v), prepared by dissolving vanillin powder in MeOH, and then 2.5 mL of a 72% (v/v) H_2SO_4 solution was added. After 10 minutes of incubation at 70°C, the mixture was quickly cooled with ice water to room temperature. The mixture's absorbance at 560 nm was determined using a UV-Vis spectrophotometer (Biochrom Libra S50, United Kingdom) with a 10 mm cuvette path length. Escin and extraction solvents served as the standard and controls, respectively. Milligrams of EE per gram of DW were used to express TSC.

Saponin extraction efficiency (SEE)

The SEE of the *H. helix* extracts was analysed based on the previous report[26]. After the dried sample (0.2 g) was extracted in solvent (20 mL) at the optimal conditions, a filtration step was carried out using filter paper in order to recover the extracts. Residual parts continued to be extracted under the same conditions until the obtained extracts did not react with reagents (four times). All the extracts were gathered and adjusted to a total volume of 80 mL. Finally, TSC was determined according to the above method. SEE was calculated according to the following equation.

$$SEE = \frac{SCE}{SCC} \times 100$$

Where SEE is saponin extraction efficiency (%); SCE and SCC are saponin content in the extracts at different extraction parameters and control (mg EE/g DW).

Total phenolic content (TPC)

The reported method illustrated the process as comprising the following steps[25,27]: First, 2.5 mL of the Folin-Ciocalteu 10% (v/v) reagent was combined

with 0.5 mL of the *H. helix* before waiting for 6 min for the mixture to stabilise. The mixture was combined with 2 mL of Na_2CO_3 7.5% (w/v), and then left in the dark for an hour. Following that, a UV-Vis spectrophotometer operating at 765 nm was used to measure the absorbance of the mixture. In this experiment, the standard and controls were various extraction solvents and gallic acid. Milligrams of GAE per gram of DW were used to express TPC.

Total flavonoid content (TFC)

According to the former report with some modifications[25], 2 mL of distilled water, 0.15 mL of $NaNO_2$ 5% (w/v) and 0.5 mL of the extract were combined. After being incubated in the darkroom for 6 min at room temperature, the mixture was then added 0.15 mL of $AlCl_3$ 10% (w/v). After 6 min incubation without the light, the mixture was supplied with 2 mL of NaOH 10% in w/v and 0.7 mL of distilled water, and then kept at ambient conditions without the light for 15 min. The mixture's absorbance was determined at 510 nm using a UV-Vis spectrophotometer. Extraction solvents and Catechin were corresponding to controls and standards. Milligrams of CE per gram of DW were used to express TFC.

Thin layer chromatography (TLC)

According to the former report[28], the study was conducted on pre-coated silica gel 60 F254 aluminium sheets with the size of 10 × 10 cm as a stationary phase, while a mobile phase involved ethyl acetate, methanol, acetone and anhydrous formic acid (30:20:20:4 in v/v). A line was constructed 1.5 cm above the bottom plate. The standard and sample solutions were spotted on the line; the amount of injection was fixed at 20 µL. A 20 by 20 cm twin trough glass chamber was used for the experiment. After the mobile phase migrates to a place, which is 0.5 cm of top plate, the plate is removed from the chamber. The plate was sprayed with a 20% H_2SO_4 solution in EtOH solution, and then heated to 105°C for 10 min and analysed under a UV light at 365 nm to determine the presence of Hederacoside C in the optimal extract. The different concentrations of Hederacoside C were utilised for generating the calibration curve.

ABTS radical scavenging capacity (ARSC)

The ARSC analysis of the *H. helix* is conducted following the published reports[29,30]. Initially, a stock solution was prepared by combining ABTS+ aqueous solution (7.4 mM) with $K_2S_2O_8$ aqueous solution (2.6 mM) in a ratio of 1:1 (v/v). The mixture was incubated without light for 12 hours under ambient conditions before being. To make a working solution that had an absorbance of 1.1±0.02 at 734 nm before measuring ARSC, the stock solution (1 mL) was combined with MeOH (60 mL). The extracts (0.15 mL) are then mixed with this solution (2.85 mL). Three hours of room temperature storage of the mixture occurred in a dark area. The mixture's absorbance was calculated utilising a UV-Vis spectrophotometer at 734 nm. Trolox and extraction solvents served as controls and standards, respectively. Milligrams of TE per gram DW were used to express the results.

DPPH radical scavenging capacity (DRSC)

The previously described method[25,29] was used to test the DRSC of the *H. helix* extracts. Initially, a stock solution was prepared with 0.024% DPPH (w/v), diluted in MeOH, and kept at -20°C. Before performing DRSC, the stock solution (1.0 mL) was diluted MeOH (45 mL) to achieve an absorbance of 1.1±0.02 at 515 nm. In the next step, the working solution (2.85 mL) and the extract (0.15 mL) were mixed and incubated for 3 hours at room temperature without light. The mixture's absorbance was examined at 515 nm using a UV-VIS spectrophotometer. Trolox and extraction solvents were utilised in this experiment as controls and standards, respectively. Milligrams of TE per gram of DW were used to represent the results.

Ferric reducing antioxidant power (FRAP)

The FRAP analysis of the *H. helix* was conducted based on the depicted reports[29,30]. The FRAP reagent included the combination of Reagents A, B, and C (10:1:1 in v/v). Reagent A was a 300 mM acetate aqueous buffer solution with a pH of 3.6, Reagent B was a mixture of TPTZ (10 mM) and HCl (40 mM), and Reagent C was an aqueous solution of $FeCl_3.6H_2O$ (20 mM). 0.15 mL of the extract and 2.85 mL of the FRAP reagent were combined for the FRAP reaction, which took place in a darkened room at ambient temperature for 30 min. Trolox and extraction solvents were used as the standard and the controls, respectively. The mixture's absorbances were calculated at 593 nm using a UV-Vis spectrophotometer. Milligrams of TE per gram of DW were used to express the results.

Statistical analysis

All measurements were obtained in triplicate (n = 3), and data are presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA with Tukey's HSD post-hoc test (SPSS Version 22.0, Chicago, IL, USA), and significance was accepted at $p < 0.05$.

Results and Discussion

Influence of solvent on phytochemical compounds and antioxidant activity of *H. helix*

Table 1 shows that the highest TSC was observed in the *H. helix* extract using 100% MeOH (40.44 mg EE/g DW), which significantly differed from the other extracts. Whereas, the lowest TSC was obtained when extracting the *H. helix* in 100% Ace (13.33 mg EE/g DW). Saponins are polar compounds, including triterpene or steroid aglycones with one or more sugar chains, and they prefer to dissolve well in polar solvents. These results highlight the significant influence of solvent polarity on the extraction efficiency of TSC from the *H. helix* L, which tends to dissolve well in polar protic solvents, particularly MeOH, rather than polar aprotic solvents such as acetone. The former study revealed that the TSC from the *P. amarus* was declined in order into corresponding solvents: MeOH, EtOH, EtOAc, water, DCM (dichloromethane), and MeCN (acetonitrile)[15], whereas the EtOH extract of papaya leaf contained the highest TSC compared to its MeOH, acetone and water extracts[25]. Although SEE is one of the key factors that assesses the effects of extraction variables on the extraction yield, research on SEE remains limited. Table 1 demonstrates that, among the seven solvents, the highest SEE (55.54%) was achieved with 100% MeOH. Except for the MeOH and acetone extracts, there were no significant differences to be observed in the *H. helix* extracts. It is worth noting that although MeOH is not as healthy as water or EtOH, it is widely used due to its low boiling point, which facilitates the concentration of extracts. Moreover, MeOH recovery can reduce costs and environmental impact by recycling it for other purposes instead of disposal. Therefore, MeOH is a suitable choice for extracting TSC in the subsequent experiments.

Considering TPC and TFC, water and 50% Ace were significantly preferred in TPC collection from the *H. helix* at 3.46 and 3.45 mg GAE/g DW, respectively (Table 1). The findings emphasise the significant impact of solvent choice on the extractability of TPC in the *H. helix*. Polar protic solvents are preferred over polar aprotic solvents to obtain the highest TPC. Furthermore, water is a "green" solvent, offering advantages in terms of cost, human health, and environmental impact, making it a promising solvent for extracting TPC from *H. helix.* The findings in Table 1 indicate that pure MeOH and pure EtOH extracts of *H. helix* possessed the largest TFC (0.25 and 0.22 mg CE/g DW, respectively), whereas the *H. helix* extract in 50% EtOH had the lowest TFC (0.04 mg CE/g DW). There were no significant differences in TFC of the *H. helix* extracts dissolved in water, 100% Ace, 50% Ace, and 50% MeOH. The former work observed a gradual decrease in TPC extracted from the *P. amarus* when using water, MeOH, EtOH, MeCN, DCM, and EtOAc[15]. According to Sabino *et al.*, dynamic maceration using medium- to high-polarity solvents in a single liquid phase resulted in superior extraction yields, with acetone (19.64%) and isopropanol (14.66%) performing best. In contrast, for Soxhlet extraction, ethanol (23.85%) and acetone (20.03%) achieved the highest yields[16]. In addition, another study found that

Table 1 — TSC, SEE, TPC and TFC of *H. helix* extracts acquired by seven solvents

Solvents	TSC (mg EE/g DW)	SEE (%)	TPC (mg GAE/g DW)	TFC (mg CE/g DW)
Water	28.89 ± 1.38^{b}	39.68 ± 1.90^{b}	3.46 ± 0.17^{a}	0.14 ± 0.01^{b}
100% MeOH	40.44 ± 0.59^{a}	55.54 ± 0.80^{a}	1.83 ± 0.07^{c}	0.25 ± 0.01^{a}
50% MeOH	26.36 ± 1.96^{b}	36.20 ± 2.69^{b}	2.54 ± 0.12^{b}	0.11 ± 0.01^{b}
100% EtOH	24.53 ± 5.72^{b}	33.69 ± 7.85^{b}	0.93 ± 0.05^{d}	0.22 ± 0.02^{a}
50% EtOH	30.61 ± 1.89^{b}	42.04 ± 2.59^{b}	2.58 ± 0.18^{b}	0.04 ± 0.01^{c}
100% Ace	13.33 ± 0.79^{c}	18.32 ± 1.09^{c}	0.78 ± 0.15^{d}	0.12 ± 0.04^{b}
50% Ace	27.27 ± 1.19^{b}	37.45 ± 1.64^{b}	3.45 ± 0.40^{a}	0.12 ± 0.02^{b}

Abbreviations include TSC (total saponin content), SEE (saponin extraction efficiency), TPC (total phenolic content), TFC (total flavonoid content), EE (escin equivalents), GAE (gallic acid equivalents), CE (catechin equivalents), and DW (dry weight). Means marked with different letters indicate significant differences ($p < 0.05$).

the aqueous extract of *S. aromaticum* had the highest TFC, followed by the methanol, ethyl acetate, and chloroform extracts[31]. Thus, the feasibility of extracting phytochemical substances from plant materials is greatly influenced by the choice of solvent.

Table 2 illustrates that the *H. helix* expressed the significantly strongest antioxidant capacity based on ARSC, DRSC and FRAP assays, when it was extracted in water and 50% Ace. In contrast, 100% EtOH and 100% Ace extracts displayed the weakest ARSC, DRSC and FRAP. This finding reveals a positive correlation between TSC, TPC and TFC of the *H. helix* with its antioxidant capacity. In comparison with other investigations, the *P. trimera*'s MeOH and aqueous extracts had the greatest ARSC, followed by its acetonitrile, ethyl acetate, and hexane extracts, while the *P. trimera* root extract in MeOH had a greater FRAP compared to this plant's extracts in water, acetonitrile, ethyl acetate, and hexane[32]. In addition, the strongest DRSC for the *P. amarus* was obtained by extracting the plant in water, followed by extracting it in MeOH, EtOH, EtOAc, MeCN, and DCM[15]. In summary, the extraction solvent had a significant impact on how easily *H. helix*'s antioxidant capacity may be extracted.

Influence of extraction techniques on phytochemical compounds and antioxidant activity of *H. helix*

Table 3 illustrates that CE demonstrated the highest potential for collecting saponin compounds from *H. helix* (42.48 mg EE/g DW for TSC and 58.34% for SEE), with a significant difference compared to UAE and MAE. This result highlights the significant impact of the extraction method on the extractability of phytochemical compounds from the *H. helix*. Although UAE and MAE are advanced methods with higher extraction yields than CE, the choice of extraction method should consider the characteristics of the desired compounds. It is important to note that sensitive constituents in the *H. helix* may be prone to damage due to the specific mechanisms of UAE and MAE. Additionally, CE offers advantages to manufacturers in terms of lower production costs and easy setup without requiring complex equipment, facilitating scale-up. Table 3 also shows no significant difference in the TPC and TFC of *H. helix* extracts obtained through CE and UAE, whereas MAE had the lowest efficiency in collecting TPC and TFC from *H. helix*. In contrast to this finding, a previous study demonstrated that MAE was more effective in extracting TSC and TPC from the *P. trimera* root and cacao pod husk compared to UAE and CE. However, there were no significant differences in

Table 2 — Antioxidant capacity of *H. helix* extracts acquired by seven solvents

Solvents	ARSC (mg TE/g DW)	DRSC (mg TE/g DW)	FRAP (mg TE/g DW)
Water	9.57 ± 0.54^{a}	3.48 ± 0.33^{a}	5.22 ± 0.76^{ab}
100% MeOH	7.00 ± 0.56^{cd}	1.93 ± 0.42^{bc}	4.06 ± 0.24^{b}
50% MeOH	7.79 ± 0.07^{bc}	1.36 ± 0.12^{bc}	4.09 ± 1.00^{b}
100% EtOH	4.79 ± 0.20^{e}	0.90 ± 0.10^{c}	1.55 ± 0.05^{c}
50% EtOH	7.85 ± 0.09^{bc}	2.10 ± 0.18^{b}	4.77 ± 0.55^{ab}
100% Ace	5.79 ± 0.40^{de}	1.22 ± 0.44^{bc}	1.56 ± 0.52^{c}
50% Ace	9.13 ± 0.97^{ab}	3.58 ± 0.76^{a}	6.28 ± 0.88^{a}

ARSC, DRSC, FRAP, TE, and DW correspond to ABTS radical scavenging capacity, DPPH radical scavenging capacity, ferric reducing antioxidant power, trolox equivalents, and dry weight, respectively. Means assigned different letters indicate significant differences (p <0.05).

Table 3 — TSC, SEE, TPC and TFC of *H. helix* acquired by three extraction methods

Extraction methods	TSC (mg EE/g DW)	SEE (%)	TPC (mg GAE/g DW)	TFC (mg CE/g DW)
CE	42.48 ± 1.00a	58.34 ± 1.38a	2.24 ± 0.05a	0.27 ± 0.01a
UAE	38.23 ± 2.71b	52.51 ± 3.73b	2.25 ± 0.10a	0.28 ± 0.01a
MAE	38.17 ± 0.50b	52.42 ± 0.69b	1.88 ± 0.06b	0.22 ± 0.02b

Abbreviations include TSC (total saponin content), SEE (saponin extraction efficiency), TPC (total phenolic content), TFC (total flavonoid content), EE (escin equivalents), GAE (gallic acid equivalents), CE (catechin equivalents), DW (dry weight), CE (conventional extraction), UAE (ultrasound-assisted extraction), and MAE (microwave-assisted extraction). Values with different letters indicate significant differences (p <0.05).

TFC of *P. trimera* root acquired by using CE, UAE, and MAE[32,33].

The antioxidant activity of *H. helix* was assessed using ARSC, DRSC, and FRAP assays (Table 4). Of which, those produced using UAE and CE did not significantly differ from each other and were significantly stronger than those using MAE (p <0,05). However, the opposite side was true for the *P. amarus* and *P. trimera*, for which MAE enhanced the highest antioxidant capacity of the extracts compared to other extraction methods[15,32]. According to research on the effects of extraction method on DRSC of Hawthorn's organs (flowers and berries), maceration produced the strongest DRSC for the berries, while UAE produced the higher DRSC for the flower part compared to other methods. Furthermore, the maximum FRAP values for Hawthorn blossoms and berries were obtained when extracting in UAE and maceration, respectively[34]. The results show that the target compounds' characteristics had a significant effect on the choice of extraction method.

Regarding the impact of extraction mechanisms on the extractability of phytochemical and antioxidant properties, during UAE, acoustic cavitation promotes the disruption of plant cell walls, leading to an enhanced mass transfer rate. The collapse of cavitation bubbles adjacent to the plant surface produces microjets that rupture cellular tissues and facilitate the liberation of the desired compounds[21,2], while MAE operates through the direct absorption of microwave energy by the sample, which is subsequently converted into heat. The resulting ionic migration increases solvent permeability within the plant matrix and promotes efficient solvation of the desired constituents[35]. However, in this instance, CE has displayed as a better technique in recovering phytochemical compounds and antioxidant activity from *H. helix* than UAE and MAE.

Influence of methanol concentration on phytochemical compounds and antioxidant activity of *H. helix*

Table 5 describes that the TSC of *H. helix* increased when dissolved in MeOH at various concentrations from 60 to 80% (v/v). Once the utilised MeOH concentration exceeded 80%, TSC dropped. In particular, 80% MeOH produced the highest SEE of *H. helix* (72.50%), while pure MeOH produced the lowest SEE (63.20%). Although there was no significant difference in SEE between MeOH concentrations from 60 to 90%, the ideal MeOH concentration could be between 70 and 80% or 80 and 90%. The results indicate that the TSC in the *H. helix* increases with solvent polarity until a certain point where the dissolution of its saponin components becomes unsuitable. This finding is supported by the former research[36], which demonstrated that the TSC of *B. falcatum* increased up to 70% MeOH and then significantly decreased when pure MeOH was used. Thus, 80% MeOH is considered for TSC extraction from the *H. helix* for further experiment.

Table 4 — Antioxidant capacity of *H. helix* extracts acquired by three extraction methods

Extraction methods	ARSC (mg TE/g DW)	DRSC (mg TE/g DW)	FRAP (mg TE/g DW)
CE	7.04 ± 0.17a	1.76 ± 0.12a	3.80 ± 0.21a
UAE	7.32 ± 0.22a	1.87 ± 0.09a	3.95 ± 0.11a
MAE	6.36 ± 0.15b	1.14 ± 0.03b	3.17 ± 0.15b

ARSC, DRSC, FRAP, CE, UAE, MAE, TE, and DW correspond to ABTS radical scavenging capacity, DPPH radical scavenging capacity, ferric reducing antioxidant power, conventional extraction, ultrasound-assisted extraction, microwave-assisted extraction, trolox equivalents, and dry weight, respectively. Means assigned different letters indicate significant differences (p <0.05).

Table 5 – TSC, SEE, TPC and TFC of *H. helix* extracts acquired by different methanol concentrations

Solvent concentration	TSC (mg EE/g DW)	SEE (%)	TPC (mg GAE/g DW)	TFC (mg CE/g DW)
60% MeOH	49.15 ± 2.27^{ab}	67.51 ± 3.11^{ab}	4.02 ± 0.03^{a}	0.19 ± 0.01^{c}
70% MeOH	52.27 ± 1.73^{ab}	71.79 ± 2.37^{ab}	3.59 ± 0.06^{b}	0.18 ± 0.01^{c}
80% MeOH	52.78 ± 2.21^{a}	72.50 ± 3.03^{a}	3.37 ± 0.07^{c}	0.18 ± 0.01^{c}
90% MeOH	51.80 ± 0.63^{ab}	71.15 ± 0.87^{ab}	2.82 ± 0.09^{d}	0.22 ± 0.01^{b}
100% MeOH	46.02 ± 3.71^{b}	63.20 ± 5.09^{b}	2.12 ± 0.11^{e}	0.26 ± 0.03^{a}

Abbreviations include TSC (total saponin content), SEE (saponin extraction efficiency), TPC (total phenolic content), TFC (total flavonoid content), EE (escin equivalents), GAE (gallic acid equivalents), CE (catechin equivalents), and DW (dry weight). Values with different letters indicate significant differences (p <0.05).

The MeOH concentration had an inverse relationship with the TPC of *H. helix* (Table 5), which declined from 4.02 to 2.12 mg GAE/g DW when the concentration increased from 60 to 100%. In contrast, the TFC of *H. helix* was increased as MeOH concentration increased. A similar pattern was observed in the TPC of *M. sylvestris* L. and *V. myrtillus* L.[37], when different EtOH concentrations were used. Both samples exhibited an increase in TPC until reaching a specific EtOH concentration. The *M. sylvestris* L. showed the highest TPC with 80% EtOH, while *V. myrtillus* L. preferred 50% EtOH. Similarly, the TFC of *T. serpyllum* L. increased with EtOH concentration until reaching the maximum content at 50% EtOH.

The results in Table 6 indicate that the antioxidant capacity of *H. helix* declined gradually as the MeOH concentration increased from 60 to 100%. The primary reason for this is largely due to solvent polarity and the chemical nature of antioxidant compounds. Antioxidants in plants are diverse (phenolics, flavonoids, saponins, etc.), and many of them are polar or moderately polar. MeOH is polar (polarity index of 5.1), but water is even more polar (polarity index of 9.0). Thus, a mixture of MeOH and water (e.g., 60-80%) provides a balanced polarity, which can extract a broader spectrum of antioxidants (both polar and moderately polar). Pure MeOH is less effective at solubilising highly polar compounds (such as phenolic acids), resulting in a lower antioxidant yield. Therefore, at 60-80% MeOH, extraction is more efficient, giving higher antioxidant activity. As MeOH concentration increases to 100%, fewer polar antioxidants are extracted, hence lower antioxidant activity is measured[15,38]. The former study also demonstrated that solvent concentration directly impacted the antioxidant capacity of grape stems, as measured by ARSC, DRSC, and FRAP tests. Among different concentrations of ethanol (0, 25, 50, 75, and 100%), the extract collected using 50% EtOH has displayed the strongest antioxidant activity[39]. Of these, MeOH concentration has significantly influenced the phytochemical compounds and antioxidant activity from the H. helix.

Influence of extraction temperature on phytochemical compounds and antioxidant activity of *H. helix*

Table 7 illustrates that TSC of the *H. helix* was ranked at the highest level at 50°C (52.77 mg EE/g DW for TSC and 72.48% for SEE). Afterwards, as the temperature increased to 60°C and 70°C, the TSC of this plant slightly decreased (51.06 and 49.94 mg EE/g DW, respectively). However, there was an insignificant difference between SEE of *H. helix* extracted at 50, 60, and 70°C (72.48, 70.13 and 68.59%, respectively). In addition, TPC and TFC of *H. helix* had a positive correlation with extraction temperature, which ranged from 3.40 to 3.84 mg

Table 6 — Antioxidant capacity of *H. helix* extracts acquired by different methanol concentrations

Solvent concentration	ARSC (mg TE/g DW)	DRSC (mg TE/g DW)	FRAP (mg TE/g DW)
60% MeOH	13.93 ± 0.65^{a}	7.38 ± 0.45^{a}	8.20 ± 0.38^{a}
70% MeOH	13.97 ± 0.36^{a}	5.89 ± 0.90^{b}	7.38 ± 0.14^{b}
80% MeOH	11.79 ± 0.23^{b}	5.48 ± 0.34^{b}	6.85 ± 0.20^{b}
90% MeOH	8.94 ± 0.19^{c}	3.72 ± 0.06^{c}	6.06 ± 0.25^{c}
100% MeOH	6.59 ± 0.24^{d}	1.62 ± 0.15^{d}	4.26 ± 0.13^{d}

ARSC, DRSC, FRAP, TE, and DW correspond to ABTS radical scavenging capacity, DPPH radical scavenging capacity, ferric reducing antioxidant power, trolox equivalents, and dry weight, respectively. Means assigned different letters indicate significant differences (p <0.05).

Table 7 — TSC, SEE, TPC and TFC of *H. helix* extracts acquired by four various temperatures

Temperature (°C)	TSC (mg EE/g DW)	SEE (%)	TPC (mg GAE/g DW)	TFC (mg CE/g DW)
40	48.00 ± 2.70^{b}	65.92 ± 3.70^{b}	3.40 ± 0.13^{b}	0.17 ± 0.02^{b}
50	52.77 ± 2.26^{a}	72.48 ± 3.10^{a}	3.71 ± 0.13^{a}	0.19 ± 0.01^{ab}
60	51.06 ± 1.42^{ab}	70.13 ± 1.95^{ab}	3.79 ± 0.07^{a}	0.20 ± 0.01^{a}
70	49.94 ± 1.04^{ab}	68.59 ± 1.43^{ab}	3.84 ± 0.15^{a}	0.21 ± 0.01^{a}

Abbreviations include TSC (total saponin content), SEE (saponin extraction efficiency), TPC (total phenolic content), TFC (total flavonoid content), EE (escin equivalents), GAE (gallic acid equivalents), CE (catechin equivalents), and DW (dry weight). Values with different letters indicate significant differences (p <0.05).

GAE/g DW for TPC and 0.17 to 0.21 mg CE/g DW for TFC. The previous work also examined the impact of various extraction temperatures (60, 80, 100, 120, and 140°C) on the extraction yield of the total saikosaponins of *B. falcatum* roots[36] and found that the saikosaponin content was increased as the temperature rose from 60 to 120°C before declining at 140°C. Similarly, TPC of soursop peel extract had a positive correlation with selected temperature range (25, 30, 40, 50, and 60°C)[40]. Moreover, TFC of Turkish green tea collected from three shooting periods gradually went up when they were extracted at 75, 85, and 95°C, respectively[41].

Table 8 shows that when extraction temperature went up from 40 to 70°C, ARSC and FRAP of the *H. helix* were also risen properly (11.33 to 12.81 mg TE/g DW for ARSC and 6.15 to 7.69 mg TE/g DW for FRAP, respectively), while DRSC of this plant was upwardly until extraction temperature was reached at 60°C (4.97 mg TE/g DW) before being slightly reduced at 60°C (4.94 mg TE/g DW). The results obtained in this study could be explained by the fact that the extraction temperature greatly influences the dissolvability and diffusibility of antioxidants into the solvent during extraction, leading to an increase in solutes and antioxidant capacity of the extract. The previous work observed that among the four selected extraction temperatures (25, 35, 45, 55, and 65°C), the ARSC of *C. asiatica* was obtained at the strongest level at 35°C, and then decreased at temperatures above 35°C[42]. In addition, another study showed that three out of four types of rice demonstrated higher FRAP values when extracted at 50°C, compared to extractions conducted at 30°C and 70°C[43]. Another work also illustrated that the antioxidant capacity of soursop peel extract exhibited a proportional increase with the growth of temperatures (25, 30, 40, 50, and 60°C) without significant difference based on DRSC assay[40]. In short, the *H. helix* showed an insignificant difference in antioxidant capacity at 50°C based on three assays compared to the remaining ones. Therefore, 50°C is an appropriate extraction temperature for the next experiment on the *H. helix*.

Influence of extraction time on phytochemical compounds and antioxidant activity of *H. helix*

As shown in Table 9, selected extraction durations insignificantly affect the extraction efficiency of phytochemical constituents from the *H. helix*. The highest TSC of the *H. helix* extract was acquired when the length of extraction was carried out at 30 min (52.33 mg EE/g DW for TSC and 71.87% for SEE). After that, TSC slightly declined with the expansion of the extraction duration. This reduction could result in the degradation of thermolabile compounds or the reabsorption of dissolved constituents into the residue sample that existed in

Table 8 — Antioxidant capacity of *H. helix* extracts acquired by four various temperatures

Temperature (°C)	ARSC (mg TE/g DW)	DRSC (mg TE/g DW)	FRAP (mg TE/g DW)
40	11.33 ± 0.39^{b}	4.20 ± 0.30^{b}	6.15 ± 0.39^{b}
50	12.06 ± 0.38^{ab}	4.65 ± 0.24^{ab}	6.76 ± 0.35^{ab}
60	12.52 ± 0.35^{a}	4.97 ± 0.26^{a}	7.61 ± 1.00^{a}
70	12.81 ± 0.37^{a}	4.94 ± 0.18^{a}	7.69 ± 0.59^{a}

ARSC, DRSC, FRAP, TE, and DW correspond to ABTS radical scavenging capacity, DPPH radical scavenging capacity, ferric reducing antioxidant power, trolox equivalents, and dry weight, respectively. Means assigned different letters indicate significant differences (p <0.05).

Table 9 — TSC, SEE, TPC and TFC of *H. helix* extracts acquired by five different extraction periods

Extraction time (min)	TSC (mg EE/g DW)	SEE (%)	TPC (mg GAE/g DW)	TFC (mg CE/g DW)
20	51.30 ± 1.59^{a}	70.46 ± 2.19^{a}	3.29 ± 0.11^{a}	0.15 ± 0.01^{a}
30	52.33 ± 1.69^{a}	71.87 ± 2.32^{a}	3.42 ± 0.17^{a}	0.16 ± 0.01^{a}
40	51.88 ± 1.69^{a}	71.25 ± 1.85^{a}	3.45 ± 0.03^{a}	0.17 ± 0.01^{a}
50	51.72 ± 1.61^{a}	71.03 ± 2.22^{a}	3.59 ± 0.12^{a}	0.17 ± 0.01^{a}
60	51.70 ± 0.93^{a}	71.00 ± 1.28^{a}	3.57 ± 0.09^{a}	0.16 ± 0.01^{a}

Abbreviations include TSC (total saponin content), SEE (saponin extraction efficiency), TPC (total phenolic content), TFC (total flavonoid content), EE (escin equivalents), GAE (gallic acid equivalents), CE (catechin equivalents), and DW (dry weight). Values with different letters indicate significant differences (p <0.05).

the solvent[21]. Although the *H. helix* achieved a high SEE (70.46%) at 20 min extraction, which showed no significant difference compared to other lengths of extraction, the optimal time could be more than 20 min when combined with other conditions to obtain the highest phytochemical compounds from the *H. helix*. Therefore, a 30-minute duration is suggested to utilise for the following investigation. The prior work found that the TSC of *Pu'er Tea* seeds was remarkably grown during the 80-minute extraction period. However, there was a slight decrease in the TSC in the subsequent 10 min of extraction[44].

Regarding TPC and TFC, when the extraction duration increased from 20 min to 50 min, TPC and TFC of the *H. helix* were slightly increased to reach a peak (3.59 mg GAE/g DW and 0.17 mg CE/g DW, respectively) before slightly declining at 60 min extraction. In particular, there was no change in TFC from 40 to 50 min. The reason for this may be explained by the fact that when the extraction time is extended, the dissolvability and diffusibility of solutes into the solvent reach a balance state. Similar to the depicted report, among the selected extraction durations (30, 60, 90, 120 and 150 min), TPC of *M. sylvestris* L. showed an increasing trend until reaching a peak at 90 min, while TPC of *V. myrtillus* L. reached its highest value when extracted for 60 min, but decreased as the extraction duration was extended[37]. The former study[37] also examined the influence of extraction time (30, 60, 90, 120, and 150 min) on the TPC extractability from purple-skinned greengage and quince and found that the purple-skinned greengage achieved the highest TFC when extracted for 120 min, while the quince reached this peak at a 90 min extraction duration. In short, extraction time has a significant impact on the extraction efficiency of phytochemical compounds from plant materials.

As presented in Table 10, while ARSC of the *H. helix* was slightly increased with increasing the length of extraction from 20 to 60 min (11.52 to 12.13 mg TE/g DW), while its DRSC and FRAP were raisen until extraction duration reached at 50 min (6.26 and 7.07 mg TE/g DW, respecively) before being slightly decreased at 60 min (6.19 and 6.71 mg TE/g DW, respecively). However, the antioxidant capacity of *H. helix* was insignificantly changed when extracted in these extraction durations. The previous study examined ARSC of *T. serpyllum* L. across different extraction durations (5, 15, and 30 min) and revealed that 30 min extraction of this plant exhibited the highest ARSC, although there was no significant difference as compared to 15 min extraction[45]. Additionally, another work showed that the *C. asiatica* extract has exhibited the highest DRSC at 180 min among various extraction times (60, 120, 180, 240, and 300 min). However, the extraction duration was prolonged, and there was a gradual decrease in the electron-donating activity of the extract[42]. In addition, among different extraction durations (30, 60, 90, 120, and 150 min), FRAP of *M. sylvestris* L. was achieved at the strongest level at 60 min extraction before being slightly decreased with the growth of extraction time, whereas the equivalent figure for *C. oblonga* Mill required a period of 90 min extraction to achieve the strongest FRAP[37]. Based on these findings, a duration of 30 minutes is deemed suitable for further investigations with *H. helix*.

Influence of sample to solvent ratio on phytochemical compounds and antioxidant activity of *H. helix*

Table 11 indicates that TSC of the *H. helix* was increased as the sample to solvent ratios rose from 1:20 to 1:100 (w/v) (42.63 to 52.57 mg EE/g DW). However, when the sample to solvent ratio was further increased to 1:120 and 1:140 (w/v), there was

Table 10 — Antioxidant capacity of *H. helix* extracts acquired by five different extraction periods

Extraction time (min)	ARSC (mg TE/g DW)	DRSC (mg TE/g DW)	FRAP (mg TE/g DW)
20	11.52 ± 0.37[a]	5.06 ± 0.07[a]	6.30 ± 0.11[a]
30	11.76 ± 0.38[a]	6.08 ± 0.40[a]	6.44 ± 0.29[a]
40	11.83 ± 0.40[a]	6.11 ± 0.56[a]	6.85 ± 0.21[a]
50	11.85 ± 0.48[a]	6.26 ± 0.32[a]	7.07 ± 0.31[a]
60	12.13 ± 0.77[a]	6.19 ± 0.26[a]	6.71 ± 0.21[a]

ARSC, DRSC, FRAP, TE, and DW correspond to ABTS radical scavenging capacity, DPPH radical scavenging capacity, ferric reducing antioxidant power, trolox equivalents, and dry weight, respectively. Means assigned different letters indicate significant differences (p <0.05).

a slight drop in TSC of the *H. helix* (50.36 and 49.62 mg EE/g DW, respectively). In addition, the greatest SEE of *H. helix* was achieved at a 1:100 (w/v) ratio (72.21%), which was not a significant difference compared to other ratios, except at a 1:20 (w/v) ratio (58.55%). Moreover, the TPC of the *H. helix* was increased as the sample to solvent ratio increased from 1:20 to 1:120 (w/v) and reached at 4.50 mg GAE/g DW. However, TPC was slightly decreased at a 1:140 (w/v) ratio (4.43 mg GAE/g DW), whereas TFC of the *H. helix* was slightly increased as the sample to solvent ratio increased from 1:20 to 1:140 (w/v) (0.16 to 0.23 mg CE/g DW). The role of kinetic energy in the extraction process is crucial. As thermal energy is added, the kinetic energy of molecules increases, causing them to move faster. This enhanced motion increases the possibility of collisions with plant cell walls and each other, leading to improved extraction efficiency. Therefore, at a fixed extraction duration, higher kinetic energy may be required for extracting TSC from the *H. helix* at the ratios of 1:120 and 1:140 (w/v). Similarly, in the case of Pu'er tea seeds, the TSC increased significantly with an increasing sample-to-solvent ratio until it reached a peak at a 1:8 (w/v) ratio. Beyond this ratio, the TSC was gradually decreased as reported in a former work[44]. In addition, the reported work found that increasing the sample to solvent ratio from 1:10 to 1:30 (w/v) led to an increase in TPC and TFC from *T. serpyllum* L[45]. In summary, the sample-to-solvent ratios of 1:40 and 1:60 (w/v) offer many benefits for manufacturers and the environment, whereas ratios of 1:100 (w/v) are an appropriate choice for further optimisation when paired with other factors.

As shown in Table 12, ARSC of the *H. helix* reached a peak at a 1:80 (w/v) sample to solvent ratio (15.74 mg TE/g DW) before being slightly decreased at 1:100, 1:120 and 1:140 (w/v) (15.35, 15.34, and 15.33 mg TE/g DW, respectively). The extracts acquired ARSC at 1:20 and 1:40 (w/v) ratios (10.08 and 13.56 mg TE/g DW, respectively) were significantly lower than other ratios. A gradual increase in DRSC was observed when the sample-to-solvent ratio was raised from 1:20 to 1:120 (w/v), with corresponding values increasing from 4.49 to 6.79 mg TE/g DW. Particularly, when this plant was extracted at a 1:140 ratio, its DRSC significantly descended (4.86 mg TE/g DW).

Table 11 — TSC, SEE, TPC and TFC of *H. helix* extracts acquired by seven various ratios

Ratios (g/mL)	TSC (mg EE/g DW)	SEE (%)	TPC (mg GAE/g DW)	TFC (mg CE/g DW)
1:20	42.63 ± 0.91b	58.55 ± 1.25b	3.35 ± 0.03d	0.16 ± 0.01c
1:40	47.04 ± 0.67ab	64.61 ± 0.92ab	3.44 ± 0.01d	0.19 ± 0.01b
1:60	47.13 ± 0.70ab	64.73 ± 0.96ab	3.83 ± 0.04c	0.20 ± 0.01b
1:80	49.84 ± 0.37a	68.45 ± 0.51a	4.14 ± 0.01b	0.22 ± 0.01a
1:100	52.57 ± 1.47a	72.21 ± 2.02a	4.35 ± 0.05ab	0.22 ± 0.01a
1:120	50.36 ± 3.53a	69.17 ± 4.85a	4.50 ± 0.14a	0.22 ± 0.01a
1:140	49.62 ± 3.90a	68.15 ± 5.36a	4.43 ± 0.20a	0.23 ± 0.01a

Abbreviations include TSC (total saponin content), SEE (saponin extraction efficiency), TPC (total phenolic content), TFC (total flavonoid content), EE (escin equivalents), GAE (gallic acid equivalents), CE (catechin equivalents), and DW (dry weight). Values with different letters indicate significant differences ($p < 0.05$).

Table 12 — Antioxidant capacity of *H. helix* extracts acquired by seven different ratios

Ratios (g/mL)	ARSC (mg TE/g DW)	DRSC (mg TE/g DW)	FRAP (mg TE/g DW)
1:20	10.80 ± 0.01^{c}	4.49 ± 0.06^{d}	6.30 ± 0.24^{c}
1:40	13.56 ± 0.29^{b}	5.74 ± 0.16^{c}	7.28 ± 0.01^{b}
1:60	15.10 ± 0.34^{a}	6.18 ± 0.16^{bc}	7.98 ± 0.06^{a}
1:80	15.74 ± 0.18^{a}	6.25 ± 0.09^{abc}	8.23 ± 0.05^{a}
1:100	15.35 ± 0.03^{a}	6.41 ± 0.25^{ab}	8.26 ± 0.03^{a}
1:120	15.34 ± 0.10^{a}	6.79 ± 0.20^{a}	8.36 ± 0.38^{a}
1:140	15.33 ± 0.53^{a}	4.86 ± 0.42^{d}	8.40 ± 0.12^{a}

ARSC, DRSC, FRAP, TE, and DW correspond to ABTS radical scavenging capacity, DPPH radical scavenging capacity, ferric reducing antioxidant power, trolox equivalents, and dry weight, respectively. Means assigned different letters indicate significant differences ($p < 0.05$).

Moreover, FRAP of *H. helix* was slightly increased with increasing sample to solvent ratio (6.30 to 8.40 mg TE/g DW). However, there was a significant difference in FRAP between the extracts obtained at the different ratios, except for the 1:20 and 1:40 (w/v) ratios (6.30 and 7.28 mg TE/g DW, respectively). The prior research evaluated both ARSC and DRSC of *T. serpyllum* L. at various ratios from 1:10 to 1:30 (w/v) and found that its ARSC was increased with the growth of the sample to solvent ratio, while its DRSC was opposite with increasing ratio[45]. Additionally, the findings on the *H. sabdariffa* L. extract showed that the FRAP was dropped with rising sample to solvent ratio from 1:5 to 1:50 (w/v)[46]. Of these, the sample-to-solvent ratio directly impacts the extractability of phytochemical compounds, their bioactivities, as well as the environment and human health. Hence, the sample-to-solvent ratio should be selected as the most suitable one.

Thin-layer chromatography (TLC)

High-performance liquid chromatography (HPLC) is commonly used for analysing active substances, but its equipment can be expensive and impractical for underfunded labs. However, not all experiments require high accuracy, leading to the development of more affordable analysis tools. TLC is a cost-effective method for studying chemicals in non-volatile mixtures. It is simple, reasonably sensitive, and quick. TLC is considered a semi-quantitative analytical technique, relying on standard solutions to determine analyte concentrations. Additionally, high-performance thin-layer chromatography (HPTLC) is known as an enhanced version of TLC that automates various stages, improving resolution and allowing for more precise quantitative measurements. Nevertheless, HPTLC still requires the purchase of several tools, which can be as costly as HPLC systems.

Results of TLC analysis of standard Hederacoside C and *H. helix* extract are shown in Fig. 1. In which, the area of TLC plates of standard Hederacoside C was visualised under UV light at a wavelength of 365 nm, increasing at concentration from 100 µg/mL (0.58 cm^2) to 500 µg/mL (4.09 cm^2). The calibration curve was established from different concentrations of standard Hederacoside C (100, 200, 300, 400 and 500 µg/mL) based on the correlation between concentration (µg/mL) and area (cm^2) as shown in Fig. 2 to be $y = 0.0087x - 0.169$ ($R^2 = 0.9955$). Based on this equation, the Hederacoside C content in the *H. helix* extract under optimised extraction conditions was calculated to be 16.94 mg/g DW or 1.694% DW.

Based on the results obtained in this study, optimal extraction conditions were suggested for maximising TSC from *H. helix* using the CE method with 80% methanol as the solvent, a 1:100 (w/v) sample-to-solvent ratio, an extraction temperature of 50 °C, and an extraction time of 30 min. Under these conditions, the saponin-enriched extract from *H. helix* is a potential source for nutraceutical or pharmaceutical applications. To compare the UAE conditions for saponins from *H. helix*, the most efficient extraction conditions were 80% ethanol as the solvent, 40% ultrasound amplitude, 50°C temperature, 60 min extraction time, and a 1:20 (w/v) sample-to-solvent ratio[21]. These findings revealed that the extraction time of CE (30 min) in this study is much shorter than that of UAE (60 min).

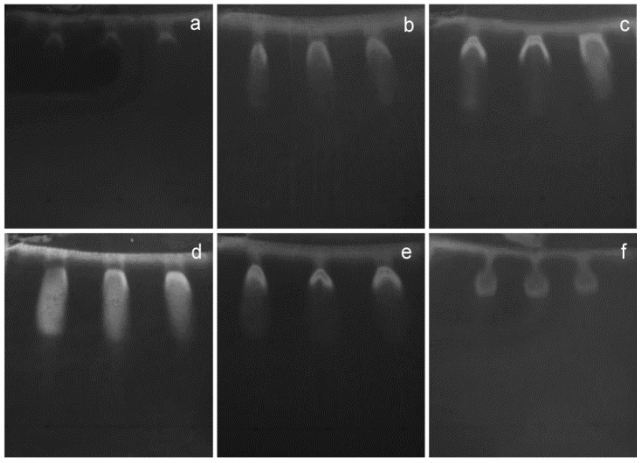

Fig. 1 — TLC plates visualised under UV light at λ=365 nm of standard Hederacoside C at, a) 100 µg/mL, b) 200 µg/mL, c) 300 µg/mL, d) 400 µg/mL, e) 500 µg/mL, and f) *H. helix* extract.

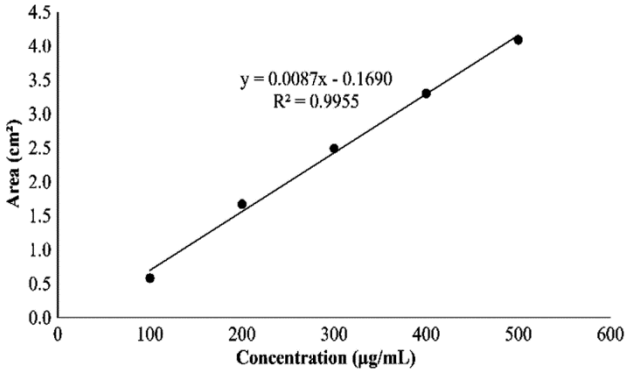

Fig. 2 — Calibration curve of standard Hederacoside C obtained by TLC analysis.

Conclusion

This study provided insight into the effect of extraction variables on the extractability of phytochemical constituents from the medicinal plant *H. helix*, which achieved the overall objective of the work. The outcome indicated that the highest TSC was achieved when *H. helix* was extracted using the CE technique with 80% methanol at a 1:100 (w/v) sample-to-solvent ratio at 50°C for 30 minutes. This finding holds significant potential application for functional food or pharmaceutical manufacturers. Moreover, additional investigation is required to enhance the optimisation of its phytochemical compounds using Response Surface Methodology and to assess their bioactivities and the mechanisms through which the active substances operate, both *in vitro* and *in vivo*.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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