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Optimization of Subcritical Fluid Extraction for Total Saponins from *Hedera nepalensis* Leaves Using Response Surface Methodology and Evaluation of Its Potential Antimicrobial Activity

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Abstract: (1) Background: *Hedera nepalensis* (Araliaceae) is a recognized medicinal plant founded in Asia that has been reported to work in antioxidant, antifungal, antimicrobial, and antitumor capacities. (2) Methods: The subcritical fluid extraction of saponin from *Hedera nepalensis* leaves and the optimum of the extraction process based on yield of saponin contents (by calculating the hederacoside C contents in dried *Hedera nepalensis* leaves) are examined by response surface methodology (RSM). Furthermore, the antimicrobial activity of the extract is tested for potential drug applications in the future. (3) Results: Based upon RSM data, the following parameters are optimal: extraction time of 3 min, extraction temperature of 150 °C, and a sample/solvent ratio of 1:55 g/mL. Under such circumstances, the achieved yield of saponin is 1.879%. Moreover, the extracts inhibit the growth of some bacterial strains (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenza*) at a moderate to strong level with inhibition zone diameter values ranging from 12.63 to 19.50 mm. (4) Conclusions: The development of such a model provides a robust experimental process for optimizing the extraction factors of saponin contents from *Hedera nepalensis* extract using subcritical fluid extraction and RSM. Moreover, the current work reveals that saponin extracts of *Hedera nepalensis* leaves exhibit a potential antimicrobial activity, which can be used as scientific evidence for further study.

Keywords: *Hedera nepalensis*; subcritical fluid extraction; response surface methodology; antimicrobial

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1. Introduction

Medicinal herbs have traditionally been used to both prevent and treat a variety of ailments. Several attempts have been made to investigate medicinal flora, and pharmacists continue to investigate the value of them across the world. *Hedera nepalensis* (Araliaceae) is a recognized medicinal plant found in Asia, mainly in Japan, Afghanistan, and the Himalayas [1,2]. According to past research, *Hedera nepalensis* contains a diverse array of natural substances, such as triterpene saponins, flavonoids, steroids, tannins, terpenoids, and phenolic compounds [3]. Particularly, triterpene saponins, with hederacoside C and alpha-hederin as major constituents, have been reported to work in antioxidant, antifungal, antimicrobial, and antitumor capacities [4–6].

A vast collection of methods can be applied for recovering bioactive compounds from natural resources, which include maceration extraction, microwave-assisted extraction, ultrasound-assisted extraction, and others [7]. Numerous downsides were found when

the usual extraction methods were applied to obtain these compounds, such as time and solvent consumption, as well as tedious and limited selectivity and/or extraction yields. Recently, subcritical fluid extraction has developed quickly as a conventional substitute for traditional extraction methods. The extraction of a liquid under pressure benefits from the enhanced solubility that happens when the solvent temperature rises. Higher temperatures improve the ability of solvents to be reconstituted for the analytes. Furthermore, raising the temperature of the solvent causes a reduction in viscosity, allowing for improved sample matrix penetration [8]. This method has been extensively utilized in recent years to extract bioactive compounds from natural materials [9–12], especially saponins [13–16]. To the best of our knowledge, there have been no publications regarding the extraction of saponins from *Hedera nepalensis* leaves by pressurized liquid extraction.

In recent years, microbial infections have increased to a great extent, and resistance to antimicrobial drugs will put the health of millions of people at risk. For mainly years, various plants have been used for daily remedies based only on traditional medicine, without adequate scientific research. With the advancement of technology in science and medicine, natural products of plants may provide a new source of antibacterial substances that might have a major impact on infectious diseases and overall community health [17,18].

The aim of our research is to optimize, by means of an experimental design using a Box–Behnken model in response surface methodology analysis, the process for extraction of saponin-rich extract from *Hedera nepalensis*. Such optimization with the proposed mathematical models will be able to properly predict the performance of the system by evaluating changes in extraction aspects such as temperature and time [19]. Moreover, the activity of these extracts against a wide range of microorganisms will be evaluated to determine the range of activity of the extract and to provide information about the antimicrobial potential of *Hedera nepalensis* extracts against infection agents.

2. Materials and Methods

2.1. Plant Materials

Leaves of *Hedera nepalensis* were harvested in Ha Giang province, Vietnam. Plant identification was carried out by the National Institute of Medicinal Material (Hanoi, Vietnam). The collected leaves were cleaned, oven-dried at 55 °C, and ground. They were stored under dry and dark conditions at room temperature. Moisture content (7.21%) was determined before further experiments.

2.2. Accelerated Solvent Extraction (ASE) Procedure

The pressured liquid extraction was performed using an ASE 350 System (Dionex, Sunnyvale, CA, USA) with a stainless-steel extraction cell. About 2 g of *Hedera nepalensis* sample was placed into an extraction cell after being uniformly mixed with the similar weight of diatomaceous earth. To avoid the powder from penetrating into the extraction bottle, a frit and a filter (Dionex) were positioned at the end of the cell. The method was as follows: solvent ethanol 50%, constant pressure 1600 psi, and other parameters (volume of used solvent, temperature, and dynamic extraction time) were chosen by performing the initial experiments (not reported here). The extract was evaporated to dryness using a rotary evaporator at 50 °C. The extract was dried using a rotary evaporator set to 50 °C. The dried extract was then diluted in 50 mL of methanol and filtered through a 0.45 µm filter for HPLC analysis.

2.3. HPLC Analysis

The saponin analysis of the extracts was conducted using HPLC as per our reported procedure [20]. Applied to this analysis, hederacoside C, a major saponin in *Hedera nepalensis*, was used as the marker for quality control of the products. The Shimadzu SPD-20A system (C18 column; 250 mm × 4.6 mm, 5 µm) (Shimadzu Co., Ltd., Kyoto, Japan) was used for HPLC analysis. A mixture of acetonitrile–0.02% phosphoric acid solution was used as the mobile phase. The composition of the mobile phase was: 0–25 min, 20–60%

acetonitrile; 25–30 min, 60–100% acetonitrile. Other running conditions included the detection wavelength (210 nm), the flow rate (1 mL/min), the injection volume (20 mL), and the column temperature (25 °C).

The percentage of hederacoside C in the portion of *Hedera nepalensis* leaves taken was calculated as follows:

$$X(\%) = \frac{\mathrm{C} \times \mathrm{V} \times \mathrm{P}}{\mathrm{m} \times (100 - \mathrm{W}) \times 10}$$

C: the concentration of analyzed compound in the sample solution from the calibration curve equation (μg/mL); V: volume of the sample solution (mL); m: weight of *Hedera nepalensis* leaves taken to prepare the sample solution (mg); W: the moisture of *Hedera nepalensis* dried leaves (%); P: purity of standard (%).

2.4. Experimental Design and Statistical Analytic

Response surface methodology (RSM) is the pattern of the design and analysis of testing, modeling procedures, and optimization approaches that utilize experimental data to obtain an approximated operational correlation between a response target and a set of proposed variables. Specifically, RSM is an empirical method created to find the best response within the individual variations among the parameters. RSM is a statistical-based technique and is a potent experimental design instrument that acknowledges the execution of whole systems [21].

Box–Behnken is a spherical design, with a centrally positioned point and middle points at the edges of the cube; it does not contain any points on the outermost corners of the cube. Application of this design was used for optimization of numerous extraction procedures, and the number of experiments was appropriately selected [22].

Design-Expert 11 (State-Ease Inc., Minneapolis, MN, USA) was used to generate the experimental designs, a statistical analysis, and regression model. In this experiment, a three-level-three-factor BBD was employed to establish the best combination of extraction variables to produce saponin-rich extract from *Hedera nepalensis*. Volume of used solvent, temperature, and dynamic extraction time were optimized by means of an experimental design. Initial experiments (not reported here) were performed to choose the experimental area for each parameter. The temperature range was confined to the 200 °C maximum system operating temperature. The range of the selected factors is reported, and three independent variables, namely volume (A), temperature (B), and time (C), were chosen, as shown in Table 1. The particle size was kept below 1.4 mm to make it easier for experimental work [23].

Table 1. Independent process variables, range and levels used for Box–Behnken design.

Independent Variable	Factors	Coded Levels		
		−1	**0**	**+1**
Volume used (mL)	A	80	100	120
Extraction temperature (°C)	B	140	170	200
Extraction time (min)	C	3	6	9

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1}^{3} \beta_{ij} X_i X_j$$

Y is the response function (yield of saponin content (%)), β_0 is the constant, and β_i, β_{ii}, and β_{ij} are the coefficients of the linear, quadratic, and cross-product terms, respectively. Accordingly, X_i and X_j are levels of the independent variables.

The analysis of variance (ANOVA) tables were created, and the effect and regression coefficients of individual linear, quadratic, and interaction terms were defined. Then, optimal conditions were counted from the final model and verified by an actual experiment attempt.

2.5. Antimicrobial Assay

2.5.1. Preparation of Test Organism Cultures

Bacterial strains: The antibacterial effectiveness of the extracts was evaluated using six bacterial strains that cause respiratory illnesses. Three strains of Gram-positive (*Staphylococcus aureus* ATCC® 25923, *Streptococcus pneumoniae* ATCC® 49619 and *Streptococcus pyogenes* ATCC® 12344) and three strains of Gram-negative (*Haemophilus influenza* ATCC® 49247, *Klebsiella pneumoniae* sub sp. *pneumoniae* ATCC® 13883 and *Pseudomonas aeruginosa* ATCC® 27853) bacteria were obtained from The Global Bioresource Center (American Type Culture Collection).

2.5.2. Inoculums Preparation

The agar plates were incubated at 37 °C and the colonies formed on them were counted after 24 h. A tryptic soy agar plate (TSA) was used for *S. aureus*, *S. pyogenes*, *K. pneumoniae*, and *P. aeruginosa*; a blood agar plate (BAP) was used for *S. pneumoniae*; and a chocolate agar plate (CAP) was used for *H. influenzae*. Using a spectrophotometer, the bacterial growth was collected using 5 mL of sterile saline water, and the wavelength was tweaked at 580 nm and diluted such that the turbidity value of the standard of 0.5 MFU (McFarland Units) corresponded to a culture density of about 1.5×10^8 cells/mL.

2.5.3. Qualitative Antibacterial Activity by Disc Diffusion Assay

Antibacterial activity of the extract was performed using the disc diffusion method. Dimethyl sulfoxide (DMSO) was used to reconstitute the extracts to reach a concentration of 200 mg/mL (final DMSO concentration not more than 5% *v/v*). After that, 60 µL of these test samples were loaded over preformed wells (8 mm in diameter) on top of the agar medium suitable for each strain. Then, 20 µg/disc of amoxicillin (Sigma) or cefotaxime (Sigma) were used as positive controls, and were loaded into filter paper discs (6 mm in diameter). For negative control, DMSO was used at a final concentration. The plates were incubated at 37 °C for 24 h. The inhibition zone surrounding the disc was measured by a ruler and was considered to be an indication of antibacterial activity: the larger the zone of inhibition, the more potent the bioactivity.

2.5.4. Quantitative Antibacterial Activity by Minimum Inhibitory Concentration

The in vitro antibacterial activity of optimum extract of *Hedera nepalensis* was found by determination of minimum inhibitory concentration (MIC). *S. pneumoniae*, *S. pyogenes*, and *H. influenzae* were investigated. The MIC value of the extract was settled as the lowest concentration that fully inhibited bacterial growth after 24 h of incubation at 37 °C. The MIC value was determined by a two-fold serial dilution technique in suitable culture media. The MIC was tested in the concentration range of 0.078–5 mg/mL. Then, 1 µL of bacterial suspension was added and cultured for 24 h at 37 °C with a density of ~1×10^4 CFU/mL to the surface of the agar plate. The agar plates were incubated at 37 °C and detected for counts of colonies growing on agar plates after 24 h. The MIC is the lowest concentration of antimicrobial agents, which stopped the visible growth of bacteria on the agar plate. Antibiotics (amoxicillin) at 0.0312–32 µg/mL and DMSO, a dissolving solvent, were used as positive controls and negative control, respectively.

3. Results and Discussion

3.1. Optimization of Extraction Using RSM

BBD designs were used in this study to explore the influence of independent factors, such as extraction temperature (140–200 °C), extraction time (3–9 min), and volume of used solvent (80–120 mL) of the *Hedera nepalensis* extracts by ASE technique. To analyze the merged effects of these factors, experiments were executed for different patterns of the parameters using statistically designed experiments (Table 2).

Table 2. Experimental results for the response value.

Std	Run	Factor A: Volume (mL)	Factor B: Temperature (°C)	Factor C: Time (min)	Response: Yield of Saponin Contents (%)
4	1	120	200	6	0.836
5	2	80	170	3	1.244
14	3	100	170	6	1.420
11	4	100	140	9	1.423
3	5	80	200	6	0.458
2	6	120	140	6	1.466
1	7	80	140	6	1.638
8	8	120	170	9	1.437
12	9	100	200	9	0.448
10	10	100	200	3	0.672
15	11	100	170	6	1.702
13	12	100	170	6	1.621
9	13	100	140	3	1.865
6	14	120	170	3	1.860
7	15	80	170	9	1.757
16	16	100	170	6	1.757
17	17	100	170	6	1.679

Table 2 shows that saponin contents from extracts varied from 0.448% to 1.865%. By applying multiple regression analysis methods, the predicted response for the extraction yield of saponin for *Hedera nepalensis* extract can be obtained and given as the second-order polynomial equation in Equation (1):

$$Y = +1.64 - 0.06 \times A - 0.50 \times B - 0.07 \times C - 0.03 \times A^2 - 0.50 \times B^2 - 0.03 \times C^2 + 0.14 \times A \times B - 0.23 \times A \times C + 0.05 \times B \times C \quad (1)$$

where Y is the predicted response and A, B, and C are the test variables: volume of used solvent (mL), temperature (°C), and time (min), respectively. The F-test was employed to determine the statistical significance of Equation (1), and the analysis of variance (ANOVA) for the response surface quadratic model is presented in Table 3.

Table 3. ANOVA for quadratic model results.

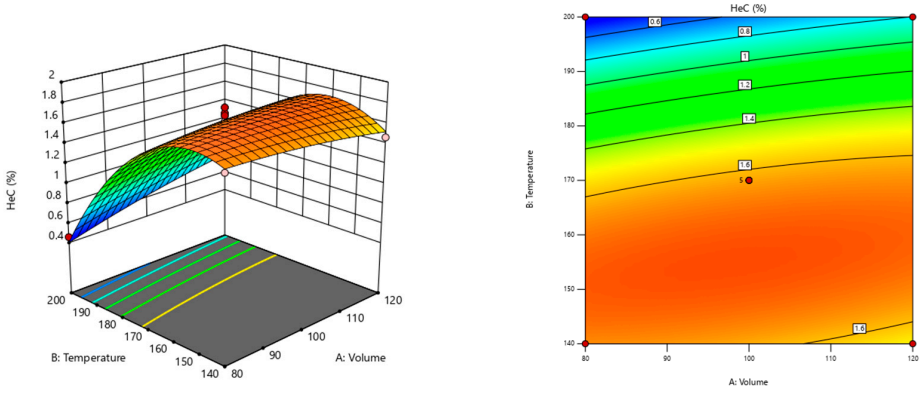
Response 1: Yield.						
Source	**Sum of Squares**	**df**	**Mean Square**	***F*-Value**	***p*-Value**	
Model	3.46	9	0.3841	17.22	0.0006	significant
A-Volume	0.0316	1	0.0316	1.41	0.2731	
B-Temperature	1.98	1	1.98	88.66	<0.0001	
C-Time	0.0414	1	0.0414	1.86	0.2154	
AB	0.0758	1	0.0758	3.40	0.1078	
AC	0.2191	1	0.2191	9.82	0.0165	
BC	0.0118	1	0.0118	0.5292	0.4906	
A^2	0.0043	1	0.0043	0.1916	0.6747	
B^2	1.07	1	1.07	48.03	0.0002	
C^2	0.0036	1	0.0036	0.1615	0.6997	

Table 3. *Cont.*

Response 1: Yield.						
Source	**Sum of Squares**	**df**	**Mean Square**	***F*-Value**	***p*-Value**	
Residual	0.1561	7	0.0223			
Lack of Fit	0.0884	3	0.0295	1.74	0.2969	Not significant
Pure Error	0.0677	4	0.0169			
Cor Total	3.61	16				
	Fit Statistics					
Std. Dev.	0.1493		R^2		0.9568	
Mean	1.37		Adjusted R^2		0.9012	
C.V.%	10.90		Predicted R^2		0.5794	
			Adeq Precision		13.4584	

The regression model's analysis of variance (ANOVA) reveals that it is very significant, as evidenced by the Fisher's F-test with a very low probability value [(Pmodel > F) = 0.0006]. The determination coefficient R^2 and the multiple correlation coefficients R can be used to assess the model's quality. The R^2 of 0.9568 reasonably settled with the adjusted R^2 of 0.9012 (both > 0.8), which showed that the model had a strong connection between the experimental data and data anticipated by the model. The closer the *R* (multiple correlation coefficient) values are to one, the stronger the correlation between the experimental and projected values. The value of R^2 (0.9568) suggests a good relationship between the experimental and anticipated response levels. Table 3 also shows that the linear coefficients (B) and quadratic coefficients (AC; B^2) were statistically significant with *p*-values < 0.05. Therefore, B, AC, and B^2 were variables that affected saponin extraction efficiency. The lack-of-fit test assesses the model's inability to reflect data in the experimental domain at locations not included in the regression. The non-significant result of lack-of-fit (>0.05) indicates that the quadratic model is statistically significant for the response, and thus may be employed in additional investigations.

The 3D response surface and 2D contour line were used to characterize the impacts of independent variables and their synergy with the yield of the saponin-rich extract. In the response surface and contour plots, extraction yield was obtained along with two continuous variables while the other variable was fixed constant at its zero level (center value of the testing ranges). It was clear that extraction yield was sensitive to minor alterations of the test variables. These graphs were drawn by imposing two other variables at their zero level, which are shown in Figure 1.



(**a**)

Figure 1. *Cont.*

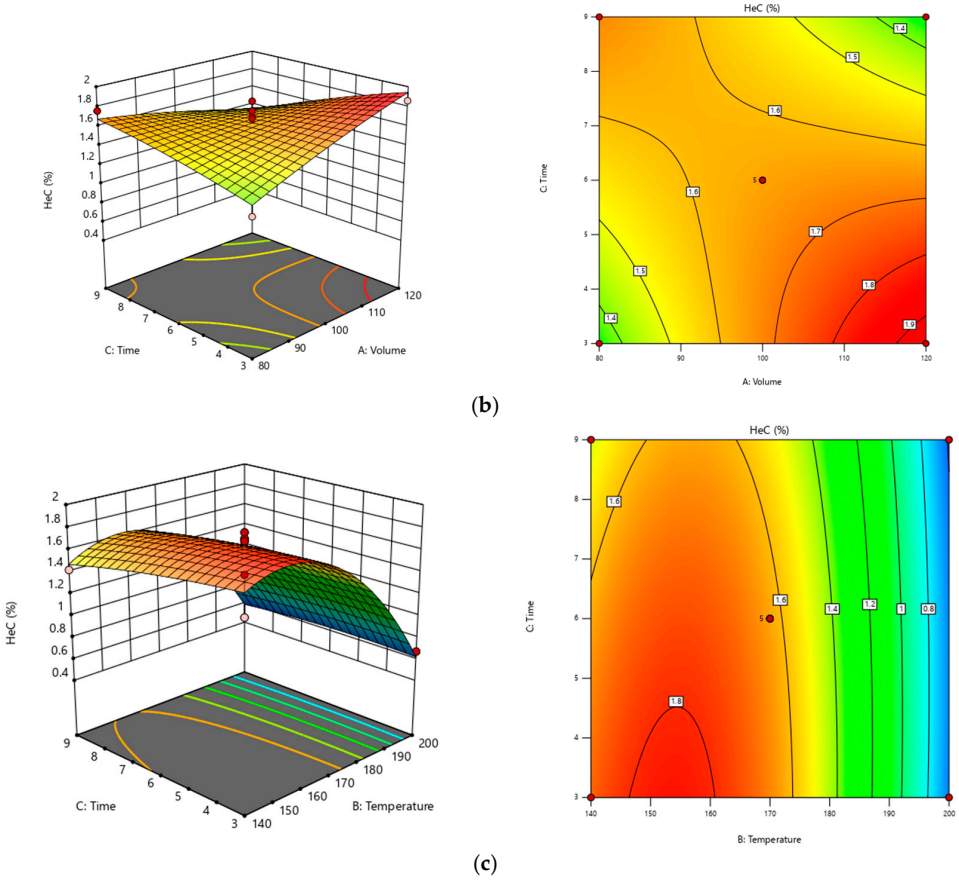


Figure 1. Response surface and contour plots for factors influencing yields of saponin extraction include: (**a**) temperature and volume; (**b**) volume and time; and (**c**) temperature and time.

The extraction yield of saponin compounds was better when the extraction temperature increased from 140 to 170 °C, but noticeably decreased when the temperature was prolonged. Because molecular mobility is accelerated with the rising temperature, these higher temperatures resulted in greater extraction efficiency. Furthermore, at higher temperatures, not only did the solvent's dissolving capability improve, so did the decrease in surface tension and solvent viscosity, which enhanced the mass transfer rate and, as a result, the availability of bioactive chemicals for extraction [24]. Although higher temperatures have a significant beneficial impact on extraction yield, they cannot be raised indefinitely. When the temperature exceeds a certain point, saponin degradation may occur in the thermal processes, reducing extraction effectiveness. This finding is consistent with earlier research demonstrating that saponin is a thermolabile substance and that high temperatures can reduce saponin extraction efficiency [25].

Figure 1b,c indicate a growing trend for saponin content when the extraction period is raised to 3 min, and a minor reduction when the duration is increased to 9 min. In this case, a longer extraction period resulted in a higher saponin percentage yield. This might be because the solute and solvent interacted with each other for a longer time. A longer contact duration enhanced mass transfer in the system. Excessive extraction time, on the other hand, is unnecessary because the solvent and sample would be in full equilibrium after a given time, based on Fick's second diffusion law. By then, the efficiency of extraction procedure would have slowed [26].

Figure 1a,b depict the interaction between extraction yield and solvent ratio. The extraction yield was observed to improve when the solvent increases. However, statistical analysis revealed that the solvent (80–120 mL) had little substantial influence on the overall saponin concentration of the extracts (Table 2). The increased concentration gradients obtained with higher solvent-to-sample ratios aid in the motion of saponins from the

interior of the material to the surface and eventually into the solvent phase, allowing less saponin to remain trapped inside the sample [27].

By examining the maximum created by the X and Y coordinates, the optimal values of the variables may be determined. Responses were temperature range of 150 °C, extraction time of 3 min, and a solvent level of 110 mL. After a batch of tripled experiments, the efficiency of extraction was 1.879% ± 1.343%, which was close to the prediction of the model (1.899%). These points were found within the experimental ranges, suggesting that these analytical approaches might be utilized to find the best conditions.

It is common practice to validate the fitted model to ensure that it gives a sufficient approximation of the real system. The residuals from least squares are critical for determining the appropriateness of a model. A normality assumption check was performed by creating normal probability plots of the residuals (Figure 2a). As the residual plots resembled a straight line, the normality conditions were met. The plots of residuals vs. expected responses are shown in Figure 2b. In general, the residuals spread randomly over the display, meaning that the variances of the original observations of these figures are constant for all values of extract yield. Figure 2c depicts the relationship between the actual and anticipated values of saponin yield, proving that the model was appropriate based on the minimum residuals and the residuals' close relationship to the diagonal line. As a result, the empirical models may be used to describe the extraction yield and bioaccessibility of total saponin by the response surface.

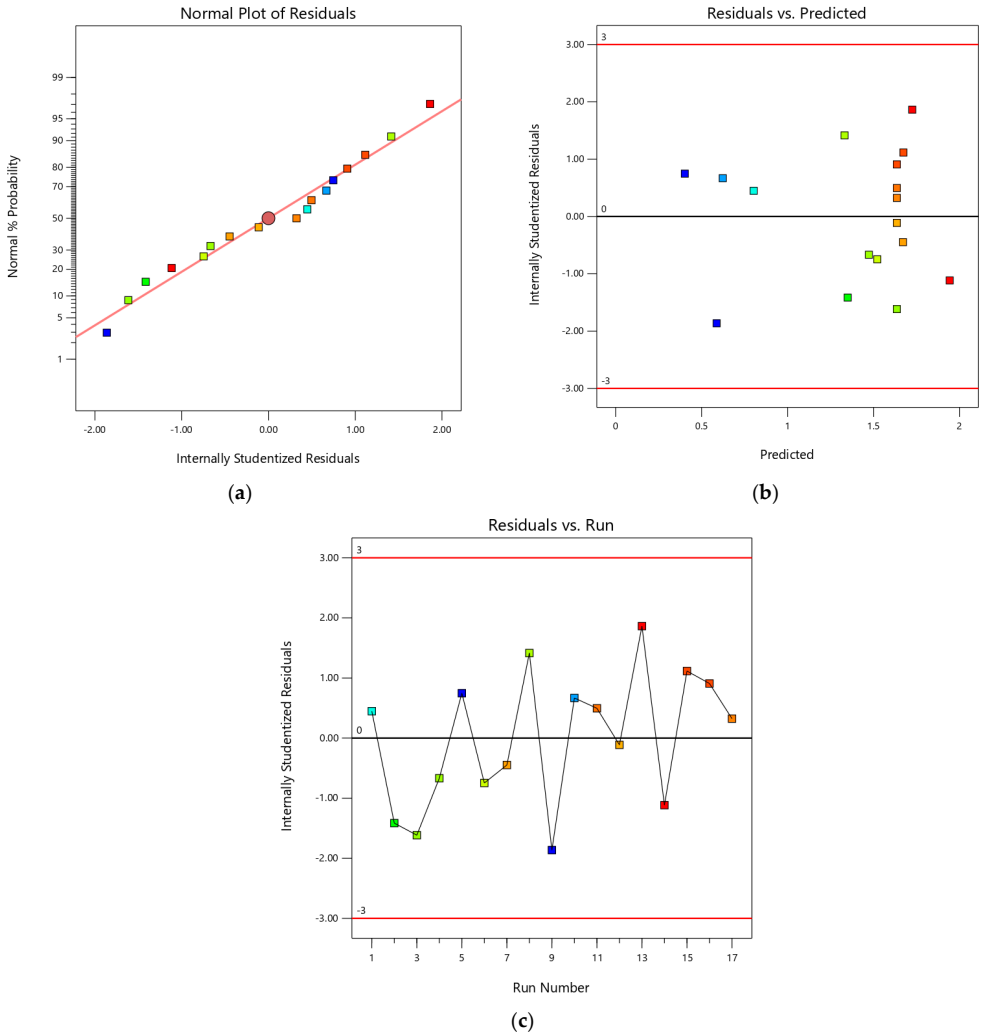


Figure 2. (**a**) The normal probability plot of residuals, (**b**) plot of internally studentized residuals versus experimental runs, and (**c**) plot of predicted and actual values.

Extraction is the first part of the process of isolating the active components from natural material and usually needs plenty of work to achieve significant success. For example, research on *Hedera nepalensis* using convenient methods such as maceration or percolation will take at least 24 h, or even days [5,28]. Thus, the process of study will take more time simply for the extraction and preparation of the sample process. Recently, with the help of modern technology such as ASE and RSM, the extraction method is considerably more rapid and can be calculated to accomplish the desired target under acceptable conditions. As in the experiments conducted above, the time needed to finish the extraction method was below ten minutes for each sample, a significant decrease in extraction times compared to conventional extraction methods reported in the literature. The results of our experiments are in agreement with the outcome of previous studies, where the production of bioactive compounds in medicinal herbs was carried out using similar techniques: the pressurized liquid method achieved a reasonable extraction efficiency in less time using less solvent [29–31]. Additionally, the natural products business is very interested in optimizing the extraction of natural plants to develop higher-quality goods. Response surface methodology (RSM) is a powerful tool for studying the interactions between variables and improving processes or products in which several variables might affect the outputs. The RSM model developed in this study enables the optimal parameters to be applied not only to laboratory tests, but it also has the potential to be applied at an industrial scale [32,33].

3.2. Antibacterial Activity

The antimicrobial activity of the extracts from *H. nepalensis* leaves was investigated by the disk diffusion method to determine the antibacterial ability of the extracts against some bacterial strains associated with respiratory diseases (Table 4). The results show that the extract of *H. nepalensis* leaves inhibited the growth of 3/6 types of bacteria, such as *S. pyogenes*, *S. pneumonia*, and *H. influenza*. Moreover, the extract inhibited the growth of three bacterial strains at a moderate to strong level with inhibition zone diameter values ranging from 12.63 to 19.50 mm.

Table 4. Antibacterial activity of the extracts from *H. nepalensis* leaves on several strains of bacteria related to respiratory diseases.

Microorganism		Zone of Inhibition (mm)			
		Control	***Hedera nepalensis* Extract**	**Amoxicillin**	**Cefotaxime**
Gram (+)	*S. aureus*	-	-	29.23 ± 0.03	ND
	S. pneumoniae	-	19.33 ± 0.09	45.60 ± 0.06	ND
	S. pyogenes	-	18.60 ± 0.06	32.10 ± 0.06	ND
Gram (−)	*H. influenzae*	-	12.63 ± 0.19	15.83 ± 0.09	ND
	K. pneumoniae	-	-	-	24.10 ± 0.10
	P. aeruginosa	-	-	-	10.60 ± 0.10

Note: "-" has no antimicrobial activity; the inhibition zone diameter, including the diameter of the paper discs, is 6 mm for the antibiotics, and the agar well is 8 mm for the test samples. ND: not determined, n = 3, Mean ± SEM.

Furthermore, the MIC values provided evidence for the inhibitory ability at low concentrations of the extract (Table 5). The lower the MIC, the more sensitivity of bacteria react to tested extract. The MIC value of the extract was 5 mg/mL for *S. pneumoniae*, while it was greater than 5 mg/mL for the remaining strains. Overall, among the bacteria tested, the *Gram* (−) group was less sensitive than the *Gram* (+) group to the extract from *H. nepalensis* leaves. This might be related to changes in bacterial characteristics, such as cell walls, the proportion of peptidoglycan, and the type of cross-linking effect of bacterial activity [34].

Table 5. MIC values of the extracts from *H. nepalensis* leaves for several strains of bacteria related to respiratory diseases.

Microorganism		MIC	
		***Hedera nepalensis* Extract (mg/mL)**	**Amoxicillin (μg/mL)**
Gram (+)	*S. pneumoniae*	5	0.0312
	S. pyogenes	>5	<0.0312
Gram (−)	*H. influenzae*	>5	2

Antibiotic resistance continues to be a concern in a number of developing and industrialized nations, presenting a substantial threat to the global health sector [35]. Due to the ineffectiveness of currently available antimicrobials for treating infectious disorders, many researchers have turned their attention to natural products as potential sources of novel bioactive chemicals [36].

In this study, the pharmacological activity of the *Hedera nepalensis* plant was confirmed by the antimicrobial activity of the extract sample that showed activity against *S. pyogenes*, *S. pneumonia*, and *H. influenza*. These results are in agreement with previous reports [1,3,37]. The antibacterial activity of the plant could be due to the presence of saponins [38,39], with active compounds such as hederacoside C in particular [40,41]. While the extract showed a significant antibacterial activity against a variety of tested bacteria, this sample only had a negligible amount of antibacterial activity against the test bacteria, as determined by their MIC values. The reason for the different performance of antibacterial activity for the sample may be related to the use of crude extracts. However, further research is necessary to determine their efficiency in suppressing the development of parasites, viruses, and/or fungus.

4. Conclusions

Accelerated solvent extraction is a sustainable and effective technique for extracting *Hedera nepalensis* leaves. The ASE followed by the RSM model is a practical method for enriching and observing saponin concentrations in *Hedera nepalensis* leaves. In the natural product extraction process, mathematical tools and models that can explain and estimate experimental data from the extraction process would be very valuable. Additionally, the current work reveals that crude extracts of *Hedera nepalensis* leaves exhibit a potential antimicrobial activity, hence providing a scientific validation of traditional techniques and supporting scientific data in favor of in vitro research.

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Abbreviations

Accelerated solvent extraction	ASE
American Type Culture Collection	ATCC
Analysis of variance	ANOVA
Blood agar plate	BAP
Box–Behnken design	BBD
Chocolate agar plate	CAP
Colony forming unit	CFU
Dimethyl sulfoxide	DMSO
Haemophilus influenza	*H. influenza*

High-performance liquid chromatography	HPLC
Klebsiella pneumoniae	*K. pneumoniae*
McFarland units	MFU
Minimum inhibitory concentration	MIC
Pseudomonas aeruginosa	*P. aeruginosa*
Response surface methodology	RSM
Standard error of mean	SEM
Staphylococcus aureus	*S. aureus*
Streptococcus pneumoniae	*S. pneumoniae*
Streptococcus pyogenes	*S. pyogenes*
Subcritical water extraction	SWE
Tryptic soy agar	TSA

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