INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

Bioactive Components of *Helicteres hirsuta* Collected in Thua Thien Hue

Dang Thanh Long[1]*, Van Thi My Le[2], Hoang Tan Quang[1], Hoang Thi Ngoc Han[3], Tong Thi Hue[1], Nguyen Thi Quỳnh Trang[4]

1 Institute of Biotechnology, Hue University, Hue city, Thua Thien Hue, 49000, Vietnam
2 University of Sciences, Hue University, Hue city, Thua Thien Hue, 49000, Vietnam
3 Department of Natural resources and Environment of Quang Tri, Dong Ha city, Quang Tri province, Vietnam
4 University of Education, Hue University, Hue city, Thua Thien Hue, 49000, Vietnam

OPEN ACCESS

Received: 24-09-2023
Accepted: 16-10-2023
Published: xx xx xxxx

Citation: Long DT, Le VTM, Quang HT, Han HTN, Hue TT, Trang NTQ (2023) Bioactive Components of *Helicteres hirsuta* Collected in Thua Thien Hue. Indian Journal of Science and Technology 16(44): 1-9.

* **Corresponding author**.

dtlong@hueuni.edu.vn

Funding: The investigation is funded by the science and technology project at Hue University with the project code DHH2021-15-17.

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: In this study, we investigate the bioactive potential of *Helicteres hirsuta Lour.* (*H. hirsuta L.*). **Methods:** *H. hirsuta* collected in Thua Thien Hue province was used. The methanolic extract of roots and leaves was studied for its biochemical components based on the High-Performance Liquid Chromatography method and also tested for antioxidant activities Mention how the test was carried out and the endpoints considered. **Findings :** The results show that some biochemical components contained in the dry materials of *H. hirsuta* leaves and roots are safe and suitable, allowing use in the extraction and collection of total flavonoids. The optimal extraction conditions using the ultrasonic bath method included a time of 50 min, a material/solvent ratio of 1:20 (g/mL), ultrasonic frequency (12 Hz), and a solvent concentration of methanol of 50% (v/v). In this condition, the highest total flavonoid content obtained from *H. hirsuta* leaves and roots were 0.841 mg CE/mL extract (leaf) and 0.416 mg CE/mL extract (root), respectively. The DPPH free radical scavenging ability at 50% concentration (IC_{50}) of leaf and root extracts varied with IC_{50} of 0.778 mL and 0.433 mL, respectively. The methanol extract from the leaf and root of *H. hirsuta* has the best inhibitory effect on the human liver cancer cell line Hep-G2 (IC_{50} leaf = 177.22 μg/mL, and IC_{50} root = 165.21 μg/mL). **Novelty:** The results of this study are valuable reference information for exploiting, using, and processing products from *H. hirsuta* trees collected in Thua Thien Hue in the future.

Keywords: Flavonoid; Helicteres hirsuta Lour.; Flavonoid; Extract; Leaf; Root

1 Introduction

The *H. hirsuta* plant produces extremely biologically active substances that are useful in the treatment of many diseases. Different parts of the plant are largely used as medicine to treat a number of diseases such as aches and pains, back pain, insomnia, and a pale complexion and enhance their health for weak hearts successfully [(1)].In recent years,

flavonoids have attracted a lot of interest for their potential to protect human health by avoiding a number of chronic illnesses, including cardiovascular disease, type II diabetes, neurodegenerative diseases, and various cancers[2].The best solvent for the extraction of flavonoid compounds and antioxidants has been demonstrated to be methanol, according to studies on flavonoid extraction in various parts of the *H. hirsuta* by Jain et al. (2014) and Pham et al. (2015) to assess the flavonoid content as well as the antioxidant activity of the Helicteres[3,4].

To date, many studies on *H. hirsuta* have been carried out in Vietnam and around the world. Several significant pharmacological properties from different parts of the *H. hirsuta* have been demonstrated thus far, including antioxidant activity[1,3–6],analgesic and anti-inflammatory actions[7],toxic and antibacterial effects on several cancer cell lines in vitro[8–12].Studying the effects of some extraction conditions and isolation of pharmaceutical ingredients from different parts of *H. hirsuta* has been of interest to many scientists[1,3,4,12]. Quang et al. (2020) researched the isolation and acquired 12 compounds from *H.hirsuta* gathered in Binh Phuoc[13], while Chin et al. (2006) studied the isolation of active components in it originating in Indonesia and obtained six lignans[14].

In order to extract flavonoids from different portions of it in Thua Thien Hue, the best circumstances needed to do so were sought after. We investigated the effects of several variables on the extraction of flavonoids from the leaves and roots of the *H. hirsuta* tree in order to identify extracts with the greatest flavonoid concentration as a starting point for evaluating their antioxidant potential.

2 Methodology

2.1 Plant materials:

H. hirsuta leaf and root samples were gathered at the Linh Mu Pagoda in Hue City's Kim Long Ward in Thua Thien Hue province. The roots and leaves are cleaned, dried in the shade, and pre-treated to get rid of pests and damaged roots and leaves. It is pulverized and put through a filter with a particle size of 1 mm before being dried at a temperature of 50°C until they are 10% moisture, then stored in a plastic box for use in subsequent studies (Figure 1).

Fig 1. Materials of *H. hirsuta*. A. Leaves; B. Roots

2.2 Method for determining some biochemical components

2.2.1 Method determining the moisture content of fresh ingredients

Based on the method of drying to a consistent weight as described by Hong, 2003[15], the moisture content of new leaves and roots of the *H. hirsuta* was calculate. Take a moisture-measuring weighing bowl, wash it, and then dry it between 100°C and 105°C so that the weight difference between the two measurements is no greater than 0.005 mg. Before assessing the moisture content, leaves and roots must be dissected. Put 5 - 10 g of medicinal herbs in a measuring bowl with a lid to measure moisture; the medicinal layer should not be any thicker than 5 mm. Once the drying oven has been opened, the weighing bowls containing the medicinal herbs are put inside and dried for one hour at a temperature of 100 to 105°C. After that, the lids are opened, the bowls are removed, placed in a desiccator to cool, covered, and weighed several times until the difference in weight between measurements does not exceed 0.005 mg. Moisture content (X%) of medicinal herbs calculated according to the following

formula 1:

$$X\% = \frac{p-a}{p}\, x\, 100 \quad (1)$$

Here: p: number of grams of test sample before drying; a: number of grams of test sample after drying; Results are rounded and accurate to 0.01; The difference in results between two simultaneous determinations is no more than 0.5%.

Method determining total lipid content : The total lipid content in medicinal herbs was determined by the Soxhlet method. Accurately weigh 5 g of sample into a 400 mL glass beaker, add 100 mL of distilled water, 60 mL of hydrochloric acid, and a few pumice stones or glass balls to regulate the boiling point. The mixture was boiled in a water bath for 15 minutes. Then, stir and cook until boiling. Cover tightly with a watch glass and simmer gently for 30 minutes. The glass surface is rinsed with boiling water (repeatedly), collected in a glass beaker, and then filtered through Whatman paper wet with cold water containing a little clean sand.

The Whatman paper containing residue is drained, spread out over the watch glass, and then dried for two to four hours in an oven set to 105°C. The lipid total content was extracted using the Soxhlet method with petroleum ether solvent for 4 hours in a spherical flask. Stop boiling, recover the petroleum ether in the flask, and put it in the oven for 30 - 40 minutes at 60°C, cooling to room temperature in a desiccator, and weigh the mass of the flask and lipid. Repeat the drying process as above until the weight remains constant or the difference in weight between two weighings is no more than 0.001 g. Total Lipid content (X) in the sample is calculated in % according to the formula 2:

$$Total\ lipid\ content\ = \left(\frac{m_1 - m_2}{G}\right) * 100\,(\%) \quad (2)$$

Here: m_1: mass of cup (g); m_2: mass of cup and mass of fat after drying (g); G: sample mass (g); Results are rounded and accurate to 0.01.

2.2.2 Method determining total protein content method

The total protein content in medicinal herbs was determined based on the Kjeldahl method. Weigh 1 g of finely ground medicinal herbs into a Kjeldahl flask with 25 mL of concentrated H_2SO_4, add 5 g of catalyst (K_2SO_4 and $CuSO_4$, ratio 9:1), and heat slowly on the stove until the solution is obtained the colorless or blue. After completely inorganicizing the sample, rinse the Kjeldahl flask with distilled water, then translate it into a 500 mL volumetric flask, and add about 10 - 15 mL NaOH 40% and a few drops of phenolphthalein, and add distilled water to make 300 mL.

Prepare a solution in a NH_3 collecting flask containing about 10 mL of Boric acid, then insert it into the system so that the tip of the condenser tube is submerged in the Boric acid solution. Start the nitrogen distillation process when the extract in the receiving flask is roughly 150 mL. Take out the collecting flask and titrate with 0.1N H_2SO_4. Total protein content is calculated according to formula 3. Accurate to 0.01%.

$$Total\ protein\ content\ = \frac{0.0014 * (V_{H2SO4} - V'_{H2SO4}) * 100 * 6.25}{m} \quad (3)$$

Here: 0.0014: Equivalent of Nitrogen; 6.26: Conversion coefficient between Nitrogen and protein; V_{H2SO4}: Number of ml of H_2SO_4 put into the conical flask; V'_{H2SO4}: Number of ml of H_2SO_4 used for titration; m: weight of test sample.

2.2.3 Methods determining total ash content

Measurement of total ash in medicinal herbs using the ashing method at 550 - 600°C. 5 g of the sample should be weighed (to within 0.1 mg) accurately and then reweighed to a constant mass. Place the sample cup in the furnace, then gradually raise the temperature to 500 - 600°C until white ash is produced. Take out the crucible and moisten the ash with water, then dry on an electric stove and then reheat the sample in a furnace at a temperature of 500 - 600°C for about an hour. Once the crucible has been removed, let it cool to room temperature in a desiccator before weighing to the nearest 0.1 mg. The difference between the results of two successive weighs cannot be higher than 0.3 mg. Repeat this process until a steady mass is reached. Total ash content (X) expressed as % mass, calculated by the formula 4:

$$X = \frac{(m_2 - m)}{(m_1 - m)}\, x\, 100 \quad (4)$$

Here: m: weight of the cup (g); m_1: weight of crucible and test sample (g); m_2: weight of the crucible and white ash after heating and weighing to a constant mass (g). Results are rounded and accurate to 0.01. The difference in results between two simultaneous determinations is no more than: 0.1%. The final result is the average of the determined results.

2.2.4 Methods extraction and determination of total flavonoid content

As stated by Vuong et al. (2013) and Pham et al. (2017) with adjustments to fit research settings [12,16], we use the powder of *H. hirsuta* in different portions (leaves and roots) to extract total flavonoids.

In a conical flask with a capacity of 500 mL and a volume of 200 mL (ratio 1:20) in a methanol solvent system with a pH = 5, precisely weigh 10 g of powder (size 1.40 mm) and shake well. The suspension was then extracted for total flavonoids using the tank ultrasound method for 30, 50, and 70 minutes with the material: solvent ratio of 1:10, 1:20, and 1:30 g/mL (w/v), respectively, and ultrasound frequency is 10, 12, 14 Hz. The extraction was done at 60°C and at levels of 30, 50, and 70 (v/v). The circumstances employed in the following experiments are determined by the outcomes of the previous experiment. The supernatant was then removed from the solution and transferred to a fresh Erlenmeyer flask using a centrifuge operating at 15000 rpm for 15 minutes. The total flavonoid content of the extract samples from each experiment was calculated using the mg equivalent of the catechin standard. The reaction to be carried out in order to ascertain the total flavonoid content is performed as follows:

According to Pham et al. (2017) [12], the total flavonoid content was calculated. Catechin standard flavonoid solution (sigma) was diluted in 70% methanol to concentrations of 45, 90, 180, 360, and 720 g/mL. In the test tube, combine 0.15 mL of $NaNO_2$, 2 mL of distilled water, and 0.5 mL of catechin solution (concentrations 45, 90, 180, 360, and 720 g/mL). Add 0.15 mL of $AlCl_3$, shake thoroughly, and let stand for another 6 minutes after adding it. Continue adding 2 mL of NaOH and 0.7 mL of distilled water, shake well, and let stand for 15 minutes. Using a UV-Vis spectrophotometer (U2900 Hitachi, Japan), the reaction solution was measured photometrically at a wavelength of 510 nm. Three times the experiment was carried out. Excel 2010 was used to record the OD data and create a calibration line. The total flavonoid content of *H. hirsuta* is determined using the calculation based on the linear regression equation of catechin.

$$M = \frac{V_1 * m_1 * n}{V_2 * m_2 * 1000} \left(\frac{mg}{g}\right) \tag{5}$$

Here: V_1: initial extract volume, m_1: total flavonoid content calculated based on the Catechin standard curve (μg/mL), n: number of dilutions, V_2: sample volume used for reaction (mL), m_2: initial sample mass (g), 1000: conversion factor from μg to mg.

2.2.5 Method High- Performance Liquid Chromatography (HPLC)

The extract dissolved (1 μg) in 500 μL of 70% methanol and 500 μL of formic acid and then vortex-mixed and centrifuged at 14000 rpm for 10 min. Finally, 10 μL supernatant was injected into the Agilent 1260 Infinity II system (Agilent Technologies, USA) for analysis with detection probe UV, column at the 30°C column temperature. The solvent mobile phase consists of 1% formic acid and 99% acetonitrile (Merck, USA).

2.2.6 Method determination of antioxidant activity

Using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) technique as reported by Long et al. (2020) [17], the antioxidant activity of the extract was assessed. The original extract was combined with 0.2 mM DPPH solution diluted in 70% ethanol and ascorbic acid solution with concentrations of 0.05, 0.67, 0.1, 0.2, 0.5, and 1 mg/mL. In each test tube, add 1 mL of 0.2 mM DPPH to 1 mL of the test sample at each dilution, shake well, and then leave in the dark for 30 minutes. The optical absorption density should then be measured at 517 nm. Similar tests were conducted using ascorbic acid as a reference. The percentage of DPPH free radical scavenging is calculated according to the formula 6:

$$\%\mathrm{SC} = \frac{\mathrm{OD_c} - \mathrm{OD_m}}{\mathrm{OD_c}} \times 100 \tag{6}$$

Here: OD_m: optical density of the experimental sample after subtracting the blank (without DPPH); ODc: blank optical density after blank subtraction (without DPPH).

The DPPH inhibition % at various concentrations should be used to build a standard curve. Using the standard curve equation ((y) = ax + b) with y = 50% to find x (x is IC_{50} has to be discovered) [18], determine the IC_{50} value (Volume of extract or ascorbic acid at which 50% of DPPH is inhibited).

2.2.7 Method cytotoxic assay

The experiment was perform to ascertain the total cellular protein content using the optical density obtained when the protein composition of the cells was stained with Sulforhodamine B (SRB, Sigma-Aldrich, USA). The method performs as described

by Long et al. (2020) [17]. The study was activity test on two cancer cell lines: Human hepatocellular carcinoma (Hep-G2) and Human lung carcinoma (SK-LU1). The percentage inhibiting cell growth is calculated through the following formulas 7 and 8:

$$\% \, Alive \, cells = \frac{[OD_{reagent} - OD_{day0}]}{[OD_{negatlvecontrol} - OD_{day0}]} \, x \, 100 \tag{7}$$

$$\% \, inhibited \, cells = 100 - \% \, alive \, cells \tag{8}$$

2.2.8 Statistical analysis

The average measurement across the three experimental replicates is used to express the experimental results. In order to compare mean values, the ANOVA analysis of variance was applied. The analyzed values are statistically significant with $p < 0.05$. All statistical analyses were performed using SPSS software (Version 20).

3 Results and Discussion

Following the application of several techniques to extract and identify certain chemical elements and flavonoid content in the roots and leaves of *H. hirsuta*, we arrived at the following conclusions:

3.1 Determining chemical components results

The results obtained with some chemical components are shown in Table 1 : Dry powder moisture of materials from the leaves and roots of *H. hirsuta* is 5.240% and 6.260%, respectively. This value is favorable for the long-term preservation of raw materials without mold, without changing the sensory experience, and is an advantageous circumstances condition for extraction of total flavonoids (Table 1).

It is possible to extract biologically active substances from the leaves and roots of *H. Hirsuta* because of their low lipid content, which is 3.270% and 2.960%, respectively. If the extract has a high lipid content, it will become viscous, making subsequent filtration and purification difficult. Lipid-containing extracts will also be unstable and have an odd smell. Roots and leaf powder both provide 5.210% and 7.580% of the total recommended daily allowance of protein, respectively (Table 1).

Table 1. Some chemical ingredients in powder from the leaves and roots of *H. hirsuta*

Name of analysis target	Unit	Result	
		Leave	Root
Dry powder moisture	%	5.240 ± 0.135	6.260 ± 0.262
Total Mineral Salts (Total Ash)		6.030 ± 0.720	7.320 ± 0.664
Protein content		7.580 ± 0.115	5.210 ± 0.149
Lipid content		3.270 ± 0.255	2.960 ± 0.198

For leaves, the total ash content (total mineral salts) is 6.030%, while for roots, it is 7.320%. According to the Vietnamese Pharmacopoeia, several medicinal powders have a total ash concentration that ranges from 5% to 18%. Therefore, the ash content of powder from the leaves and roots of *H. hirsuta* is within the allowable limit (Table 1).

3.2 Affecting some factors in the extraction process on total flavonoid content results

The results of studying the effects of several factors on the extraction process to obtain total flavonoids from the leaves and roots of *H. hirsuta* are present in Table 2 , which show that extraction time, methanol solvent concentration, materials/solvents ratio, and ultrasound intensity all affect the extraction process and collection of total flavonoids from the leaves and roots of *H. hirsuta*. The difference is significantly significant at $p < 0.05$. The flavonoid content increased between 30 and 50 minutes and decreased between 50 and 70 minutes, but the highest value was at 50 minutes (about 7.908 mg CE/g dry (leaves) and 3.615 mg/g dry (root). The total flavonoid content may decrease due to prolonged extraction time under the effect of ultrasound because more residue is obtained [19]. Similarly, at a solvent methanol concentration of 50%, the total flavonoid content reached a maximum of 7.204 mg CE/g dry (leaves) and 3.448 mg CE/g dry (root). The amount of flavonoids decreases when the methanol concentration increases to 70% (Table 2).

The data provided in Table 2 demonstrate that the material/solvent ratio 1:20 resulted in the maximum flavonoid concentration, which equals 10.131 mg CE/g dry (leaves) and 3.859 mg CE/g dry (roots), respectively. The total flavonoid

content tended to decrease when the material/solvent ratio increased to 1:30. This may be because the flavonoid content declined as the extraction process achieved equilibrium since the concentration gradient between the solute and solvent was no longer as great. Meanwhile, the total flavonoid content tends to increase in the ultrasound intensity range from 10 to 12 Hz and later decreases if the ultrasound intensity continues to increase to 14 Hz. Total flavonoid content reached the maximum value at 12 Hz, respectively 5,259 mg CE/g dry (leaves) and 2,435 mg CE/g dry (roots) (Table 2).

Research the effects of some common extraction conditions to obtain the highest flavonoid content from *H. hirsuta* leaves and roots collected in Thua Thien Hue, Vietnam using the tank ultrasound method. The research results have determined the optimal extraction conditions such as time (50 minutes) and material/solvent ratio (1:20 g/mL) in 50% methanol concentration with the help of ultrasound intensity at 12 Hz intensity. According to the research results of Pham et al., 2017 [12], the response surface method with Box–Behnken design was used to evaluate the effects of extraction time, temperature, and material/solvent on the yield of some compounds such as phenolics, flavonoids showed optimal extraction conditions including temperature 60°C, time 35 minutes with material/solvent ratio of 1: 100 g/mL using methanol 40% (v/v) as solvent. The results of this study show that although the extraction time is lower, it consumes five times more solvent than ours.

Table 2. Effect of some factors in the extraction process and collection oftotal flavonoid content in *H. hirsuta* leaves and roots

Factor		Total flavonoid content (mg CE/g dry)	
		Leave	Root
Time (min)	30	6,142^{b}	2,585 ^{b}
	50	7,908^{a}	3,615 ^{a}
	70	7,649^{a}	3,449 ^{a}
Methanol concentration (%)	30	3,178^{b}	2,764^{c}
	50	7,204^{a}	3,448^{a}
	70	4,480^{ab}	3,368^{b}
Material/solvent ratio (g/mL)	1:10	7,011^{b}	2,274 ^{b}
	1:20	10,131^{a}	3,859 ^{a}
	1:30	9,779^{a}	3,641 ^{ab}
Ultrasound intensity (Hz)	10	4,901^{a}	2,008^{c}
	12	5,259^{a}	2,435^{a}
	14	4,998^{a}	2,289^{b}

Note: Different letters in the same column indicate statistically significant differences in the sample mean with $p < 0.05$ (Ducan's test); Note used for the next tables.

3.3 HPLC analysis results

HPLC analysis results from the extract obtained under optimal conditions shown in Figure 2 show that many signal picks of compounds appear, including 25 peaks of compounds showing characteristics with retention times ranging from 0.937 to 23.582 minutes (roots) (Figure 2 A) and 24 peaks of compounds with retention times ranging from 1.167 to 24.012 minutes (leaves) (Figure 2 B).

3.4 Biological activity results

Antioxidant activity :

Before dilution into different concentration ranges to test the antioxidant activity on the DPPH system, we calculated the total flavonoid content in 1 mL of the extract. According to the findings, 1 mL of the extract contained 0.416 mg of root flavonoids and 0.841 mg of leaf flavonoids based on the mg equivalent of catechin. The greatest average values of DPPH free radical scavenging activity were found in the roots at 89.030% and in the leaves at 96.789%. *H. hirsuta* leaf and root extracts are 50% effective at inhibiting DPPH free radicals; their respective IC_{50} values are 0.778 mL and 0.433 mL (Figure 3Table 3).

Research on antioxidant activity from ingredients from EtOAc extract obtained from the stems and leaves of *H. hirsuta* species in Vietnam has been presion by Tra et al. (2021), showed that flavonols and flavonol glycosides belonging to the flavonoid group as useful agents for the antioxidative effects when tested on cells rat liver [20]. Pham et al. (2021) showed that phenolic compounds from this plant have strong antioxidant effect including DPPH free radical scavenging activity, ABTS, and ferric-reducing antioxidant [21]. Hieu et al. (2020) showed that the ethyl acetate fraction obtained from *H. hirsute* leaves showed strong

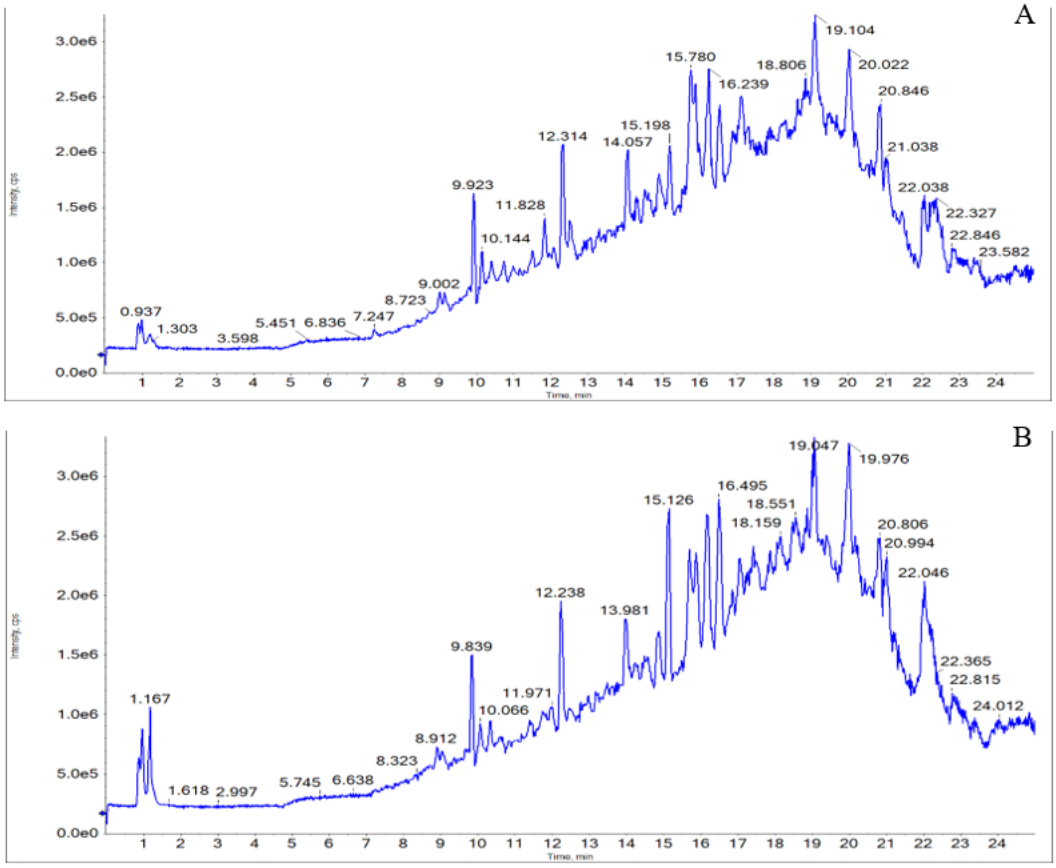

Fig 2. High Performance Liquid Chromatography (HPLC) analysis results from total extract from *H. hirsuta* leaves and roots. A: leaves and B: roots.

activity with the lowest IC_{50} = 9.50 μg/mL [22]. Methanol extracts obtained from *H. hirsuta* fin leaves and roots collected in Thua Thien Hue, Vietnam in this study showed high DPPH free radical scavenging activity, ranging from 89.030% (root) and in the leaves at 96.789%, corresponding to the flavonoid content is 0.841 mg/mL (leaves) and 0.416 mg/mL (root).

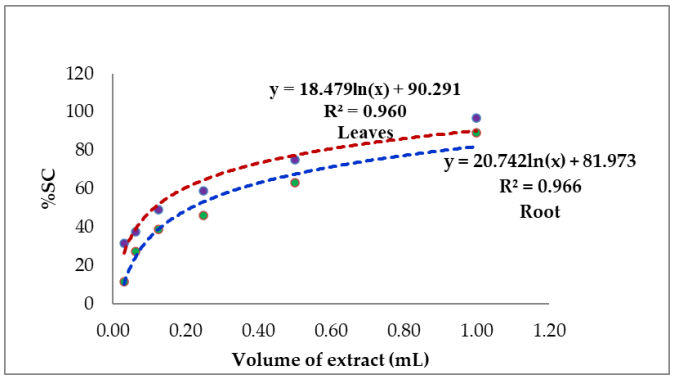

Fig 3. The DPPH free radical scavenging ability of extracts from *H. hirsuta* leaves and roots at different dilution ratios

Anticancer activity

A study on the cytotoxicity to some cancer cell lines of crude extract segment from leaf and roots of *H. hirsute* in vitro conditions shown in Table 4 shows that The ability to cause cytotoxicity on cell lines is moderate with IC_{50} values ranging from

Table 3. Total flavonoid content and percentage of DPPH free radical scavenging activity of the extract methanol of *H. hirsuta* leaves and root extracts

Sample	Extract volume (mL)	Total flavonoid content (mg CE/g dry)	SC %	IC_{50} (μg/mL)
Leave	1	0.841[a]	96.789[a]	**0.778 ± 0.004**
	0.5	0.769[b]	75.222[b]	
	0.25	0.605[c]	59.000[c]	
	0.125	0.461[d]	49.081[d]	
	0.0625	0.220[e]	37.748[e]	
	0.03125	0.120[f]	31.687[f]	
Root	1	0.416[a]	89.030[a]	**0.433 ± 0.032**
	0.5	0.319[b]	63.222[b]	
	0.25	0.218[c]	46.277[c]	
	0.125	0.101[d]	39.012[d]	
	0.0625	0.063[e]	27.147[e]	
	0.03125	0.032[e]	11.487[f]	
Acid ascorbic	-	-	-	**0.002 ± 0.001**

177.22 to 352.96 μg/mL (crude extract from leaf) and 165.21 to 274.47 μg/mL (crude extract from root), in which the best inhibitory effect on Hep-G2 with IC_{50} = 177.22 μg/mL (crude extract from leaf) and 165.21 μg/mL (crude extract from root). Thus, the crude extract obtained from the leaves and roots of *H. hirsute* showed the ability to be toxic for two cancer cell lines examined. The crude extract showed the best toxicity to the Hep-G2 cancer cell line. In the experiment, the control Ellipticine worked stably at concentrations ranging from 10 -2 - 0.4 - 0.08 μg/mL (Table 4).

Research testing toxic activity from crude extracts from different solvents of *H. hirsute* shows that the petroleum ether (PE), dichloromethane (DC), chloroform, and ethanol fractions all have the ability to cause toxicity with to Hep-G2 cancer cell line with varying degrees. The corresponding IC_{50} values are 28.29 μg/mL (petroleum ether-PE), 30.30 μg/mL (dichloromethane-DC) [12], 9.17μg/ml (chloroform), and 19.96 μg/mL (ethanol) [23]. However, the methanol (MeOH) fraction has not shown cytotoxic activity against the Hep-G2 cell line [12]. Our study shows that methanol extract from the leaf and root of *H. hirsuta* has the best inhibitory effect on the human liver cancer cell line Hep-G2 (IC_{50} leaf = 177.22 μg/mL, and IC_{50root} = 165.21 μg/mL).

Table 4. Carcinogenic effects of extract methanol from leafs and root of *H. hirsute* on cell lines

Cell lines	Leaves	Root	Ellipticine
	IC_{50} (μg/mL)		
HepG2	177.22 ± 2.17	165.21 ± 3.50	0.51 ± 0.04
SK-LU-1	253.96 ± 2.01	274.47 ± 2.40	0.45 ± 0.03

Note: The concentration of Ellipticine used in the test was 10 -2 - 0.4 - 0.08 μg/mL

4 Conclusion

With the aid of the research findings, we discovered that certain biochemical elements present in the dried materials of the leaves and roots of *H. hirsuta* collected at the Linh Mu Pagoda, Kim Long ward, Hue City, Thua Thien Hue province, provided suitable outcomes when research on medicinal plants with total flavonoid extraction was conducted. Total flavonoids could be extracted under the best circumstances under the following conditions: time (50 minutes), methanol solvent concentration (50%) material/solvent ratio (1:20 g/mL), and ultrasonic frequency (12 Hz). Under ideal circumstances, there are 0.841 mg of flavonoids in 1 mL of *H. hirsuta* leaf extract and 0.416 mg in 1 mL of root extract. The 50% efficiency of leaf and root methanol extracts at scavenging DPPH free radicals was different; the IC_{50} values were 0.778 mL and 0.433 mL, respectively. Our study shows that methanol extract from the leaf and root of *H. hirsuta* has the best inhibitory effect on the human liver cancer cell line Hep-G2 (IC_{50} leaf = 177.22 μg/mL, and IC_{50} root = 165.21 μg/mL). This is highly scientific information for further research in the exploitation and application of this medicinal plant in Thua Thien Hue, Vietnam.

References

1) Pham HNT, Van Tang Nguyen, Van Vuong Q, Bowyer MC, Scarlett CJ. Bioactive Compound Yield and Antioxidant Capacity of Helicteres hirsuta Lour. Stem as Affected by Various Solvents and Drying Methods. *Journal of Food Processing and Preservation*. 2017;41(1):e12879. Available from: https://doi.org/10.1111/jfpp.12879.
2) Vijayan K, Tsou CH. DNA barcoding in plants: taxonomy in a new perspective. *Current Science*. 2010;99:1530–1540. Available from: https://www.jstor.org/stable/24069450.
3) Jain A, Sinha P, Desai NS. Estimation of flavonoid, phenol content and antioxidant potential of Indian screw tree Helicteres isora L. *International Journal of Pharma Sciences and Research*. 2014;5(4):1320–1330. Available from: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(4).1320-30.
4) Pham H, Van T Nguyen, Vuong QV, Bowyer MC, Scarlett CJ. Effect of Extraction Solvents and Drying Methods on the Physicochemical and Antioxidant Properties of Helicteres hirsuta Lour. Leaves. *Technologies*. 2015;3(4):285–301. Available from: https://doi.org/10.3390/technologies3040285.
5) Hieu LT, Son LL, Vu H, Hoa D, Thi T. Antioxidant activity of extracts from Helicteres hirsuta Lour. Fruits in Thua Thien Hue province. *Journal of Science and Technology*. 2019;14(2):1–9.
6) Loganayaki N, Siddhuraju P, Manian S. Antioxidant activity and free radical scavenging capacity of phenolic extracts from Helicteres isora L. and Ceiba pentandra L. *Journal of Food Science and Technology*. 2013;50(4):687–695. Available from: https://doi.org/10.1007/s13197-011-0389-x.
7) Yen LTH, Ha NTT, Nga NT, Binh TV, Oanh TT. Anti-inflammatory effects and analgesic effects of liquid extracts from Helicteres hirsuta Lour. in Swiss mice. *Journal of medicine and pharmacy*. 2017;496:56–59.
8) Duyen NH, Phuoc LT. Survey of chemicalcomposition and toxicityactivity in Hep-G2 cell of Helicteres hirsuta L. *Can Tho University Journal of Science*. 2016;47:93–97. Available from: https://doi.org/10.22144/jvn.2016.605.
9) Pham HNT, Van Vuong Q, Bowyer MC, Scarlett CJ. In vitro anti-pancreatic cancer activity of HPLC-derived fractions from Helicteres hirsuta Lour. stem. *Molecular Biology Reports*. 2020;47(2):897–905. Available from: https://doi.org/10.1007/s11033-019-05180-0.
10) Pham HNT, Van Vuong Q, Bowyer MC, Scarlett CJ. Phytochemical Profiles and Potential Health Benefits of Helicteres hirsuta Lour. *The 1st International Electronic Conference on Food Science and Functional Foods*. 2021;70:43–43. Available from: https://doi.org/10.3390/foods_2020-07804.
11) Dinda B, Debnath S, Harigaya Y. Naturally Occurring Iridoids. A Review, Part 1. *Chemical and Pharmaceutical Bulletin*. 2007;55(2):159–222. Available from: https://doi.org/10.1248/cpb.55.159.
12) Pham HNT, Van Vuong Q, Bowyer MC, Scarlett CJ. Optimum conventional extraction conditions for phenolics, flavonoids, and antioxidant capacity of Helicteres hirsuta Lour. *Asia-Pacific Journal of Chemical Engineering*. 2017;12(2):332–347. Available from: https://doi.org/10.1002/apj.2076.
13) Quang DN, Pham CT, Le LTK, Ta QN, Dang NK, Hoang NT, et al. Cytotoxic constituents from Helicteres hirsuta collected in Vietnam. *Natural Product Research*. 2020;34(4):585–589. Available from: https://doi.org/10.1080/14786419.2018.1490907.
14) Chin YWW, Jones WP, Rachman I, Riswan S, Kardono LBS, Chai HBB, et al. Cytotoxic lignans from the stems ofHelicteres hirsuta collected in indonesia. *Phytotherapy Research*. 2006;20(1):62–65. Available from: https://doi.org/10.1002/ptr.1806.
15) Hong PTA. Biochemical engineering. 2003.
16) Vuong QV, Hirun S, Roach PD, Bowyer MC, Phillips PA, Scarlett CJ. Effect of extraction conditions on total phenolic compounds and antioxidant activities of Carica papaya leaf aqueous extracts. *Journal of Herbal Medicine*. 2013;3(3):104–111. Available from: https://doi.org/10.1016/j.hermed.2013.04.004.
17) Long DT, Hong HTK, Trang NTQ, Tram L, Tien NPT, Hanh NTN. Study of the Procedure for Bath Ultrasound-Assisted Extraction of Total Flavonoid from Lotus Seeds and Testing Some Biological Activities. *Indian Journal of Agricultural Research*. 2020;54(5). Available from: https://doi.org/10.18805/IJARe.A-525.
18) Marxen K, Vanselow K, Lippemeier S, Hintze R, Ruser A, Hansen UPP. Determination of DPPH Radical Oxidation Caused by Methanolic Extracts of Some Microalgal Species by Linear Regression Analysis of Spectrophotometric Measurements. *Sensors*. 2007;7(10):2080–2095. Available from: https://doi.org/10.3390/s7102080.
19) Van CV. Dictionary of Vietnamese medicinal plants. Medical Publishing. 1997.
20) Tra NT, Cham BT, Ha NTT, Anh LTT, Van Tuyen N, Son NT. Antioxidant Constituents from the Stem and Leaf of Helicteres hirsuta Loureiro. *The Natural Products Journal*. 2021;11(5):592–599. Available from: https://doi.org/10.2174/2210315510999200728131328.
21) Pham HNT, Van Vuong Q, Bowyer MC, Scarlett CJ. Phytochemical Profiles and Potential Health Benefits of Helicteres hirsuta Lour. *The 1st International Electronic Conference on Food Science and Functional Foods*. 2021;70:43–43. Available from: https://doi.org/10.3390/foods_2020-07804.
22) Hieu LT, Van Thi TT, Son LL, Nhung NM, Diep HTN, Mechler A, et al. Phenolic Contents and Antioxidant Activity of Helicteres Hirsuta Extracts. *Letters in Organic Chemistry*. 2021;18(2):128–133. Available from: https://doi.org/10.2174/1570178617999200728205824.
23) Thuy P. In vitro antioxidant and cytotoxic activities of alcohol and chloroform extract Helicteres hirsuta Lour. *Sterculiaceae Journal of Science and Technology*. 2018;4:90–92.