Volatile Organic Compounds and Antioxidant, Cytotoxic Activities of Extracts from the Leaves of *Grewia bulot*

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Abstract: *This research aims to determine the volatile compounds present in* Grewia bulot *leaf extracts and evaluate their cytotoxic and antioxidant activities. The volatile constituents of the* n*-hexane and dichloromethane extracts were identified by using gas chromatography–mass spectrometry. The main compounds identified in the former were neophytadiene (18.2%), methyl palmitate (14.4%), methyl linoleate (9.7%), β-sitosterol (4.5%), and methyl stearate (3.4%), while those in the latter were palmitic acid (9.8%), hexadecane (7.4%), octadecane (6.0%), neophytadiene (5.3%), and 2-*tert*-butoxyethanol (5.3%). The cytotoxicities of the extracts were examined against four human cancer cell lines (SK-LU-1, Hep-G2, MCF-7, and KB), while their antioxidant activities were assessed using the DPPH radical scavenging assay. The* n*-hexane and dichloromethane extracts displayed weak activity against these cancer cell lines, with IC_{50} values ranging from 90.60 ± 3.49 to 98.27 ± 2.77 μg/mL. All extracts showed antioxidant activities, and the methanol extract exhibited the strongest at an SC_{50} value of 9.39 ± 0.90 μg/mL. This is the first report on the volatile constituents and bioactivities of* G. bulot *leaf extracts, suggesting their potential application as antioxidants.*

Keywords: Grewia bulot*; cytotoxic; antioxidant; GC-MS; volatile compound*

■ INTRODUCTION

Pharmacotherapy is largely relied on natural products and structural analogs, particularly in the treatment of cancer and infectious diseases [1]. In addition, the use of medicinal plants for disease prevention and treatment has increased worldwide during the past few decades [2]. *Grewia* is a genus of evergreen shrubs/small trees of the family Malvaceae, including more than 400 species distributed mainly in the tropical and subtropical regions of Africa, Asia, and Australia. *Grewia* species is a source of food, fodder, and firewood and is notably used in traditional medicine to cure several ailments, including rheumatism, diabetes, diarrhea, and heart and blood disorders; protect the liver; cure inflammation; treat fever; and relieve pain [3-4]. A literature survey indicated that the secondary metabolites from the genus *Grewia* show diverse biological effects, such as antioxidant [5-8], antimalarial [8-9], antibacterial [10-12], antidiabetic [13], anticholinesterase [7], anticancer [8,14-16], antiplasmodial, antileishmanial, and antitrypanosomal activities [17]. Previous studies have examined the anticancer and antioxidant abilities of extracts from some plant species. Notably, the chemical constituents isolated from *Mitrephora winitii* twigs and leaves have shown significant activity against the KB and MCF-7 cancer cell lines [18]. Further, using the microwave–ultrasound-assisted method, *Moringa oleifera* leaf

extracts, which were rich in flavonoids, displayed the highest activity in a DPPH scavenging test (IC_{50} = 72.31 μg/mL) [19]. In addition, the *n*-butanol, ethyl acetate, and dichloromethane leaf extracts of *Petroselinum sativum* have demonstrated powerful free radical scavenging activity [20].

Of the 24 species of the genus *Grewia* L. distributed in Vietnam, only *G. bilamellata* has been studied [9,21-22]. Given the potential of drug discovery from plants, this work aims to examine the cytotoxic activities against four human cancer cell lines – including SK-LU-1 (human lung adenocarcinoma), Hep-G2 (human hepatocarcinoma), MCF-7 (human breast carcinoma), and KB (human oral carcinoma) – and the antioxidant activities of five leaf extracts from *G. bulot* Gagn., a species of flowering plant native to Vietnam [3,22-23].

■ EXPERIMENTAL SECTION

Materials

Specimen collection

In January 2022, *G. bulot* leaves were harvested in Quang Tri Province, Vietnam (geographical coordinates: 16°29'30.0"N 107°01'18.4"E). Dr. Nguyen Sinh Khang (Institute of Ecology and Biological Resources, VAST, Vietnam) verified the plant's authenticity. A voucher specimen (Hue.22-01) has been deposited at the Faculty of Chemistry, University of Education, Hue University, Vietnam.

Chemicals and reagents manufactures

L-glutamine, fetal bovine serum (FBS), sodium bicarbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trypsin, ethylene diamine tetraacetic acid (EDTA), trichloracetic acid, L-ascorbic acid, aspirate, dimethyl sulfoxide (DMSO), and a homologous series of *n*-alkanes (C_7–C_{40}) were obtained from Sigma-Aldrich (USA). The solvents, *n*-hexane, dichloromethane, ethyl acetate, and methanol, were also obtained from Sigma-Aldrich. Human cancer cell lines (SK-LU-1, Hep-G2, KB, and MCF-7) were generously supplied by Prof. J.M. Pezzuto (Long-Island University, USA) and Prof. J. Maier (University of Milan, Italy). Cell culture flasks and 96-well plates were obtained from Corning Inc. (USA).

Instrumentation

The volatile compositions were investigated by Gas Chromatography–Mass Spectrometry (GC-MS) method which was conducted on the Shimadzu GC-MS QP2010 Plus system. The absorbance of the cells in the cytotoxicity test was measured by the ELISA Plate Reader (USA).

Procedure

Solvent extraction process

G. bulot dried leaves (4.2 kg) were firstly powdered and then extracted with methanol (5 times, 5.0 L each) at room temperature. The resulting extract was concentrated under low pressure to afford 175.42 g of a black solid extract, with a yield of 4.2% (w/w). This extract was distributed in water and then alternately partitioned with *n*-hexane, dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc) (5.0 L, 3 times each) to obtain the *n*-hexane (GBH, 29.94 g), the CH_2Cl_2 (GBD, 21.33 g), the EtOAc (GBE, 38.22 g), and the retained water (GBW, 85.93 g) layers after removing the solvents under low pressure. GC-MS was used to analyze the *n*-hexane and dichloromethane extracts.

Determination of the volatile constituents

The volatile constituents were investigated by GC-MS method conducted on a Shimadzu GC-MS-QP2010 Plus system (Japan) equipped with an Equity-5 capillary column (30 m length, 0.25 mm diameter, 0.25 μm film thickness) and a mass spectrometer (MSD QP2010 Plus). The *n*-hexane and dichloromethane extracts (1 mg, each) were diluted in a 1:100 ratio with dichloromethane, and 1 μL of the diluted solution was used for determination. The following analytical conditions were employed: a carrier helium flow rate of 1.5 mL/min, an injector and an interface temperature of 280 °C, column temperature program starting from 60 °C (2 min hold), ramping at 3 °C/min to 240 °C (10 min hold) and subsequently increasing to 280 °C at 5 °C/min (40 min hold). The samples were injected by the split-less injection mode. For mass spectrometry, acquisitions were performed in the scan mode with a mass range of m/z = 40–500 at a sampling rate of 1.0 scan/s,

using an ionization voltage of 70 eV. The retention indices (RI) of the compounds were investigated by co-injecting a homologous series of *n*-alkanes (C_7–C_{40}) under the same conditions. The compounds were determined by comparing their mass spectra with those in Wiley 7 and NIST 11 libraries from the GC-MS system, as well as relevant literature data. Data quantification of the constituents was determined by the relative peak area [24].

Cell culture

Hep-G2, LU-1, MCF-7, and KB cell lines were chosen to use in the cytotoxicity tests. Stock cultures were grown in T-75 flasks containing 10 mL of Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS), 1.5 g L^{-1} sodium bicarbonate and 2 mM L-glutamine. Media were changed every 48 h. After that, the cells were dissociated with 0.05% trypsin-EDTA, and then subcultured at 3–5 day intervals in a ratio of 1:3, and incubated at 37 °C in a humidified atmosphere of 5% CO_2.

Cytotoxicity assays

The *in vitro* cytotoxicity assay has been confirmed by the US National Cancer Institute (NCI) as a principal test for the biological evaluation and screening of substances capable of inhibiting growth or killing cancer cells under *in vitro* conditions. This test was performed based on Skehan et al. method [25]. The cells were stained with Sulforhodamine B (SRB), and the optical density (OD) value was used to investigate the total cellular protein content. The amount of SRB attached to the protein molecule is directly related to the OD value. Thus, the more cells, as well as the more protein, correspond to higher OD values. The test was conducted following these specific conditions: Trypsinization to separate and count cells in a counting chamber to customize the suitable density. Continue to provide 190 µL of cells are performed in a 96-well plate. The test sample is dissolved in 100% DMSO to obtain an initial concentration of 20 mM. Dilute the sample on a 96-well plate with cell culture medium (without FBS) into 4 concentration ranges from high to low. Diluted reagents at different concentrations (10 µL) were introduced into the prepared 96-well plate above. Wells without reagent but with cancer cells (190 µL) + DMSO 1% (10 µL) will be used as zero-day control. After 1 h, zero-day control wells of cells will be fixed with trichloracetic acid (TCA) 20%. Cells were incubated for 72 h and then proceeded to fix with TCA for 1 h, stained with the SRB at 37 °C for 30 min. After that, the cells were washed 3 times with acetic acid, and then dried at room temperature. Dissolving 10 mM unbuffered tris base into the SRB, gently shaking for 10 min, and reading the OD results at 540 nm on an ELISA Plate Reader (Bio-Rad, California, USA). The inhibition rate (IR) of the cells was calculated by the following formula: IR% = {100% − [(OD_t – OD_0)/(OD_c–OD_0)] × 100}. The test was repeated 3 times to ensure accuracy. Ellipticine solutions at the concentrations of 10, 2, 0.4, and 0.08 µg/mL were used as reference control. A solution of 1% DMSO was used as a negative sample with a final concentration of 0.05%. The concentration that inhibits 50% of growth (IC_{50}) was investigated by using TableCurve 2Dv4 software. The extract will be considered active if the value of IC_{50} is not more than 20 µg/mL, while the pure compound will be evaluated to have good activity if the IC_{50} value is less than 5 µM, as stated by the US NCI [26].

DPPH radical scavenging activity

The DPPH radical scavenging tests were carried out based on the method of Abramovič et al. [27] with some modifications. The sample is diluted with a stock solution in methanol, and then followed by diluting a range of solutions with different concentrations with double distilled water. L-ascorbic acid (Sigma) was used as a reference control. Ascorbic acid aqueous solutions at different concentrations were diluted with double distilled water. A DPPH (Sigma) 0.25 M solution was prepared by dissolving DPPH in methanol (100%). Firstly, 100 µL methanol solution of the research sample at different concentrations was placed in a 96-well plate, and then the as-prepared DPPH solutions were added to the wells with a ratio of 1:1. The control well (blank well) included water (100 µL) and DPPH (100 µL). After that, they were incubated for 30 min at room temperature. After completing the reactions, the absorbance of the solutions (OD) was measured at 517 nm. The ability to

neutralize the free radicals created from the DPPH of the test sample was determined by Eq. (1–3):

$$\%\ \text{Scavenging activities} = \frac{OD_c - OD_s}{OD_c} \times 100 \quad (1)$$

$$\text{where}: ODc = OD_{\text{well without reagent}} - OD_{\text{blank well}} \quad (2)$$

$$ODs = OD_{\text{reagent well}} - OD_{\text{blank well}} \quad (3)$$

SC_{50} value (Scavenging Concentration at 50% – concentration that neutralizes 50% of DPPH free radicals) was investigated by using TableCurve 2Dv4 software.

■ RESULTS AND DISCUSSION

By the GC-MS analysis, 33 volatile compounds were identified in the *n*-hexane leaf extract, which accounted for 74.9% (Table 1, Fig. 1). With 31.6%, fatty acid esters were the major chemical class of the identified compounds, followed by diterpenes (18.2%), steroids (6.3%), fatty acids (5.4%), triterpenoids (4.2%), alkanes (3.2%), aromatic compounds (1.4%), diterpenoids (1.4%), triterpenes (1.2%), alkenes (1.0%), alcohols (0.7%), and monoterpenoids (0.2%). As can be seen from Table 1, neophytadiene reached the highest amount of 18.2%, followed by methyl palmitate (14.4%), methyl linoleate (9.7%), β-sitosterol (4.5%) and methyl stearate (3.4%). Other components were determined including palmitic acid (2.9%), lupeol acetate (2.3%), lupeol (1.9%), oleic acid (1.9%), β-sitostenone (1.8%), methyl elaidate (1.6%), α-tocopherol (1.4%), and squalene (1.2%). Especially worth noting that our research was discovered from *G. bulot* leaf with five unknown compounds present in the *n*-hexane extract with 21.9%. Indeed, some unknown compounds were recorded with an amount greater than 1.0% at retention time of 51.13 (16.0%), 79.88 (2.9%), 79.68 (1.4%), and 45.49 (1.1%).

For the dichloromethane extract, a total of 26 volatile components were determined, which represented 87.1% (Table 1, Fig. 2). Alkanes (31.2%), fatty acids (14.6%), and alkenes (10.6%) were the main chemical classes. Dichloromethane extract was also characterized by the presence of fatty acid esters (9.4%), alcohols (6.9%), diterpenes (5.3%), diterpenoids (4.4%),

Table 1. Volatile compositions (%) of the *n*-hexane and dichloromethane extracts of *Grewia bulot* leaves

No.	RT	Compound[a]	*n*-hexane extract	Dichloromethane extract	Identification[b,c]
1	3.95	Isovaleric acid	-	0.9	MS, RI, O
2	4.78	2-tert-Butoxyethanol	0.2	5.3	MS, RI, O
3	17.4	Dodecane	0.5	3.4	MS, RI, O
4	22.4	2-Methoxy-4-vinylphenol	-	1.1	MS, RI, O
5	26.1	Tetradecane	0.7	4.9	MS, RI, O
6	31.2	Methyl laurate	0.4	-	MS, RI, O
7	31.4	Dihydroactinidiolide	0.2	-	MS, RI, O
8	32.8	Lauric acid	-	4.0	MS, RI, O
9	33.8	1-Hexadecene	-	1.3	MS, RI, O
10	34.1	Hexadecane	0.8	7.4	MS, RI, O
11	38.8	Methyl tetradecanoate	0.4	-	MS, RI, O
12	40.2	Loliolide	-	2.7	MS, RI, O
13	40.6	Unidentified	-	2.1	MS, RI, O
14	41.1	1-Octadecene	0.1	2.2	MS, RI, O
15	41.4	Octadecane	0.5	6.0	MS, RI, O
16	42.7	Phytol	1.4	4.4	MS, RI, O
17	44.1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.5	1.6	MS, RI, O
18	44.9	Methyl oleate	0.2	0.2	MS, RI, O
19	45.5	Unidentified	1.1	-	MS, RI, O
20	45.7	Methyl palmitate	14.4	3.9	MS, RI, O
21	46.8	Palmitic acid	2.9	9.8	MS, RI, O

No.	RT	Compound[a]	*n*-hexane extract	Dichloromethane extract	Identification[b,c]
22	47.8	1-Eicosene	0.6	4.7	MS, RI, O
23	47.8	Ethyl palmitate	0.6	-	MS, RI, O
24	48.0	Eicosane	0.5	4.9	MS, RI, O
25	48.8	Methyl margarate	0.6	-	MS, RI, O
26	50.9	Methyl linoleate	9.7	2.5	MS, RI, O
27	51.1	Unidentified	16.0	3.6	MS, RI, O
28	51.3	Methyl elaidate	1.6	-	MS, RI, O
29	51.5	Neophytadiene	18.2	5.3	MS, RI, O
30	51.9	Methyl stearate	3.4	0.7	MS, RI, O
31	52.1	Linoleic acid	0.6	-	MS, RI, O
32	52.2	Oleic acid	1.9	0.8	MS, RI, O
33	52.9	Unidentified	0.5	2.8	MS, RI, O
34	53.8	1-Docosene	0.3	2.3	MS, RI, O
35	54.0	Docosane	0.3	3.0	MS, RI, O
36	59.4	Unidentified	-	1.4	MS, RI, O
37	59.6	Tetracosane	-	1.6	MS, RI, O
38	63.1	Methyl octacosanoate	0.3	-	MS, RI, O
39	64.2	Heptadecyl heptadecanoate	-	2.1	MS, RI, O
40	74.4	Squalene	1.2	-	MS, RI, O
41	79.7	Unidentified	1.4	-	MS, RI, O
42	79.9	Unidentified	2.9	-	MS, RI, O
43	82.2	α-Tocopherol	1.4	-	MS, RI, O
44	85.4	β-Sitostenone	1.8	-	MS, RI, O
45	87.2	β-Sitosterol	4.5	-	MS, RI, O
46	87.5	Lupeol	1.9	-	MS, RI, O
47	89.8	Lupeol acetate	2.3	-	MS, RI, O
		Total	96.8	96.9	
		Unidentified	21.9	9.8	
		Hemiterpenoids/Acids	0	0.9	
		Alkanes	3.2	31.2	
		Alkenes	1.0	10.6	
		Alcohols	0.7	6.9	
		Aromatic compounds	1.4	1.1	
		Diterpenes	18.2	5.3	
		Triterpenes	1.2	0	
		Monoterpenoids	0.2	2.7	
		Diterpenoids	1.4	4.4	
		Triterpenoids	4.2	0	
		Steroids	6.3	0	
		Fatty acids	5.4	14.6	
		Fatty acid esters	31.6	9.4	

[a]Compound listed according to the elution order of column Equity-5; [b] Retention Index (RI) calculated using a homologous series of *n*-alkanes (C_7-C_{40}) in a capillary column (Equity-5) (see supplementary data); [c] Identification based on the matching of mass spectra (MS), retention index (RI) of the compounds with NIST11, WILEY7, Adams (2017) data libraries, along with the data in the website http://www.thegoodscentscompany.com/search2.html (accessed on 1 March 2023) (O). Area (%): is the percentage of the area occupied by the compound within the chromatogram; - Not identified

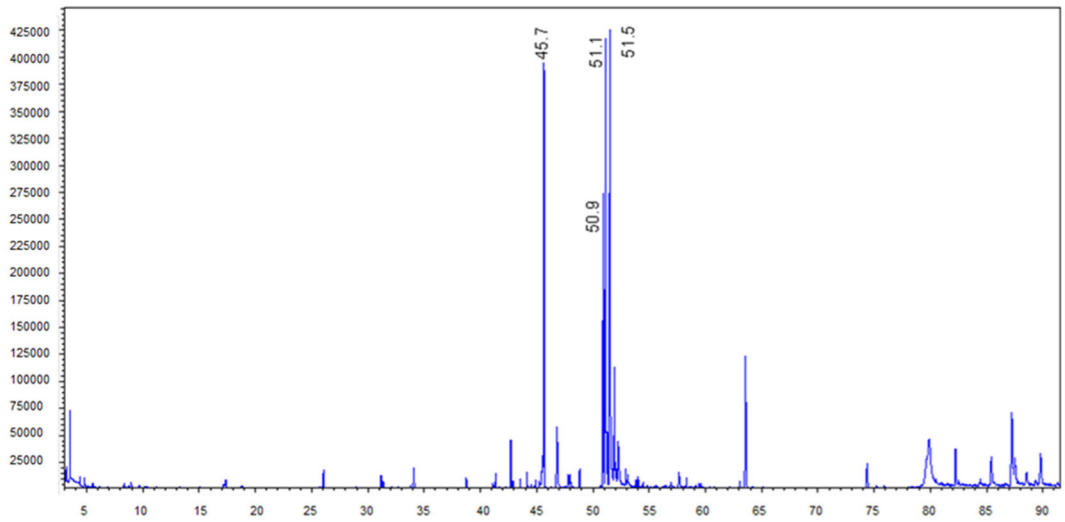


Fig 1. GC-MS data of *n*-hexane extract of *Grewia bulot* leaf

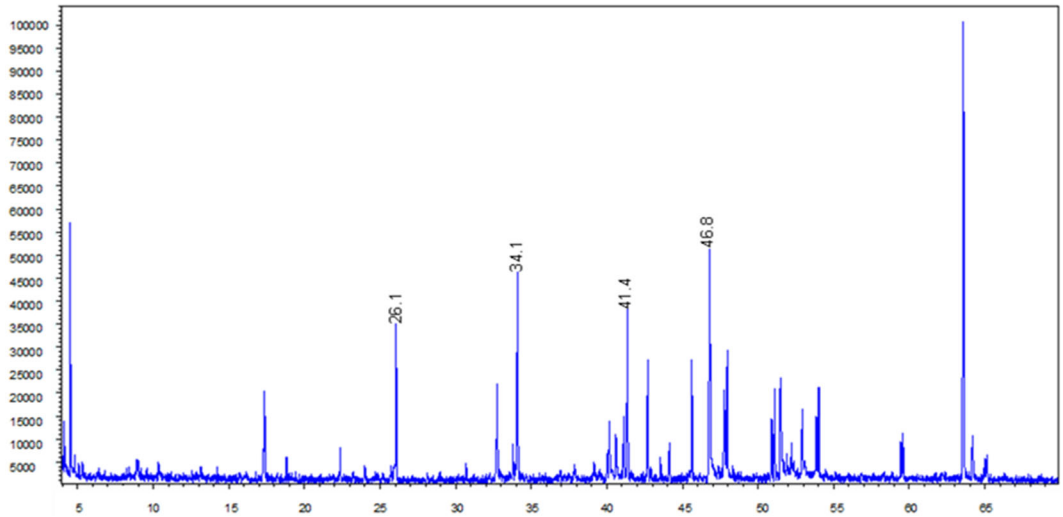


Fig 2. GC-MS data of dichloromethane extract of *Grewia bulot* leaf

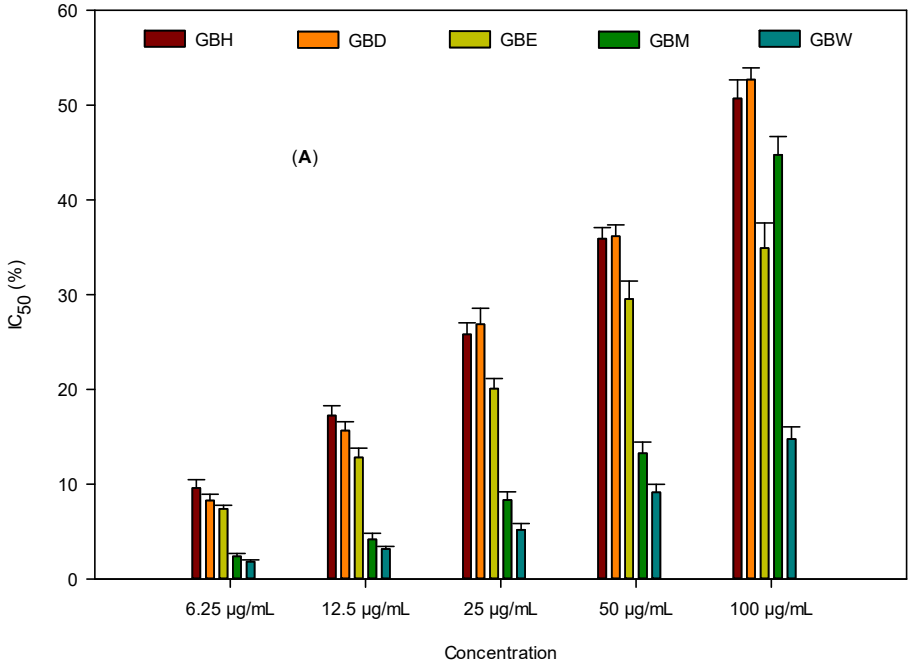
monoterpenoids (2.7%), aromatic compounds (1.7%), and hemiterpenoids/acids (0.9%). In addition, the presence of unknown compounds in leaf volatile accounted for 9.8%, including compounds at retention time 51.13 (3.6%), 52.92 (2.8%), 40.58 (2.1%), and 59.43 (1.4%). A significant amount of alkanes, fatty acids, alkenes, fatty acid esters, and alcohols were found in dichloromethane extract, accounting for 31.2, 14.6, 10.6, 9.4, and 6.9%, respectively. The principal palmitic acid (9.8%), hexadecane (7.4%), octadecane (6.0%), 2-*tert*-butoxyethanol (5.3%), tetradecane (4.9%), eicosane (4.9%), and 1-eicosene (4.7%) were determined with higher content than those of the *n*-hexane extract. Additionally, several compounds (> 1.0%) were also found in the dichloromethane extract, consisting of phytol (4.4%), lauric acid (4.0%), methyl palmitate (3.9%), dodecane (3.4%), docosane (3.0%), loliolide (2.7%), methyl linoleate (2.5%), 1-docosene (2.3%), 1-octadecene (2.2%), heptadecyl heptadecanoate (2.1%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.6%), tetracosane (1.6%), and 1-hexadecene (1.3%).

Literature survey showed that the main volatile compounds in the *n*-hexane extract from stem bark of *G. lasiocarpa* were investigated included hexadecane (10.2%), heptadecane (9.7%), tetratetracontane (7.5%), heneicosane (6.5%), hexatriacontane (5.9%), sitosterol

(5.6%), and lupeol (4.9%). Lupeol (13.7%), γ-sitosterol (7.3%), and 9*Z*-octadecenamide (6.3%) were determined in the stem bark's chloroform extract, while lupeol acetate (12.9%), 1,6-bis[(2*S*)-2-ethylhexyl]hexanedioate (9.9%), 4-((1*E*)-3-hydroxy-1-propenyl)-2-methoxyphenol (8.2%), δ-4,6-cholestadienol (5.7%), palmitic acid (5.2%), and β-sitosterol (5.2%) were identified in the methanol extract of this species [8]. Regarding the chemical constituents of *G. tenax*, sixty-three volatile compounds were identified from the fruit, in which the major compounds were acetic acid (61.0%); methylhydrazine (4.8%), 2,3-butanediol (4.1%), palmitic acid (3.5%), and 1,3-butanediol (2.4%) [28]. Generally, the volatile compositions are similar among *Grewia* species, but their content is much different to a great extent.

The cytotoxicity of *n*-hexane (GBH), dichloromethane (GBD), ethyl acetate (GBE), methanol (GBM), and water (GBW) was studied (see supplementary data). *G. bulot* extracts against the growth of the MCF-7, Hep-G2, SK-LU-1, and KB cell lines were tested using a sulforhodamine B assay (Fig. 3) [25]. The GBH and GBD samples show cytotoxic activity against some cell lines, with IC_{50} values ranging from 90.60 to 98.27 μg/mL, while the remaining samples do not exhibit such activity at the tested concentrations. According to previous reports, the essential oil extracted from the fresh leaves of *G. lasiocarpa* showed cytotoxic activity at 1 mg/mL (IC_{50} = 555.70 μg/mL) against HeLa cells, while that from the stem bark exhibited no significant activity (IC_{50} > 1000 μg/mL) [29]. Furthermore, aqueous leaf and fruit extracts from *G. asiatica* showed significant anticancer activity against liver and breast cancer, with IC_{50} values of 59.03 and 58.65 μg/mL (leaf extract) and 50.37 and 61.23 μg/mL (fruit extract), respectively, while the methanol leaf extract exhibited activity against four human cancer cell lines – HL-60, K-562, MCF-7, and HeLa – with IC_{50} values of 53.70; 54.90; 199.5 and 177.8 μg/mL, respectively [30]. In another study, the cytotoxic activity of the $CHCl_3$ fraction of *G. bilamellata* (combined leaves, twigs, and stems) against the KB cell line involved an ED_{50} > 20 μM [9]. In addition, the methanol extract of *G. hirsuta* leaves had a cytotoxic effect on the Hep-G2 cell line, with an IC_{50} value of 15.6 μg/mL, and showed a cell viability of 50.4% [15].

Furthermore, the antioxidant activities of the five crude extracts were tested by measuring their DPPH scavenging capacity, as shown in Fig. 4. All extracts exhibit antioxidant activity, with SC_{50} values ranging from



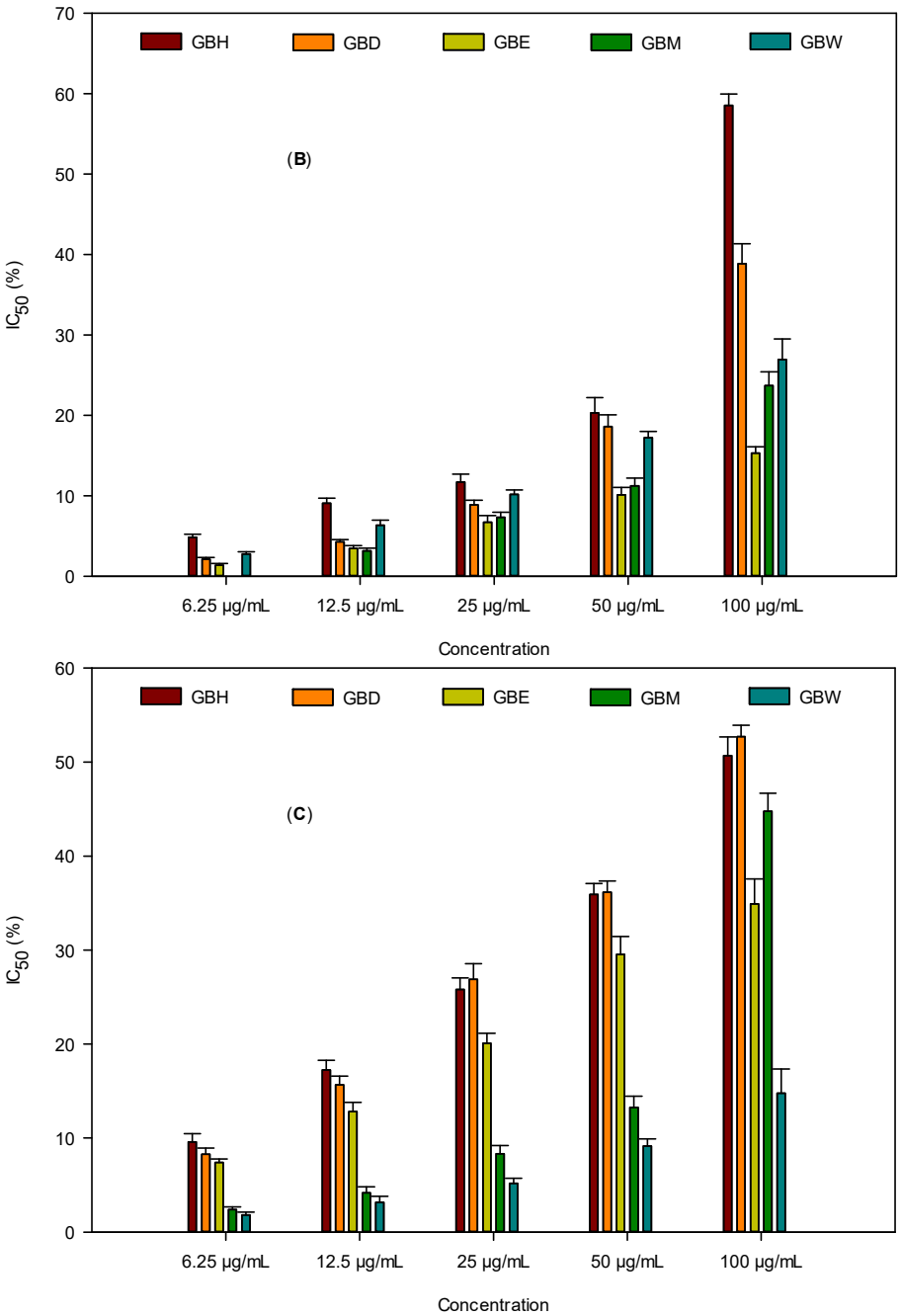


Fig 3. Effects of GBH, GBD, GBE, GBM, and GBW extracts from the *Grewia bulot* leaf on the viability of Hep-G2 (A), KB (B), MCF-7 (C), and SK-LU-1 (D), respectively. Data were expressed as a percentage of control

9.39 to 153.78 µg/mL, and the scavenging efficacy of the extracts follows the order GBM > GBE > GBD > GBW > GBH. The methanol extract (GBM) shows the strongest activity, with an SC_{50} value of 9.39 ± 0.90 µg/mL, comparable to that of the positive control, ascorbic acid (SC_{50} = 7.27 ± 0.12 µg/mL); its effectiveness as an antioxidant is attributed to its higher concentration of total phenolic compounds. As is commonly known, the antioxidant activity of an extract is directly correlated with the amount of phenolic compounds present; therefore, extracts with higher phenolic content exhibit greater antioxidant activity. This makes this plant a good

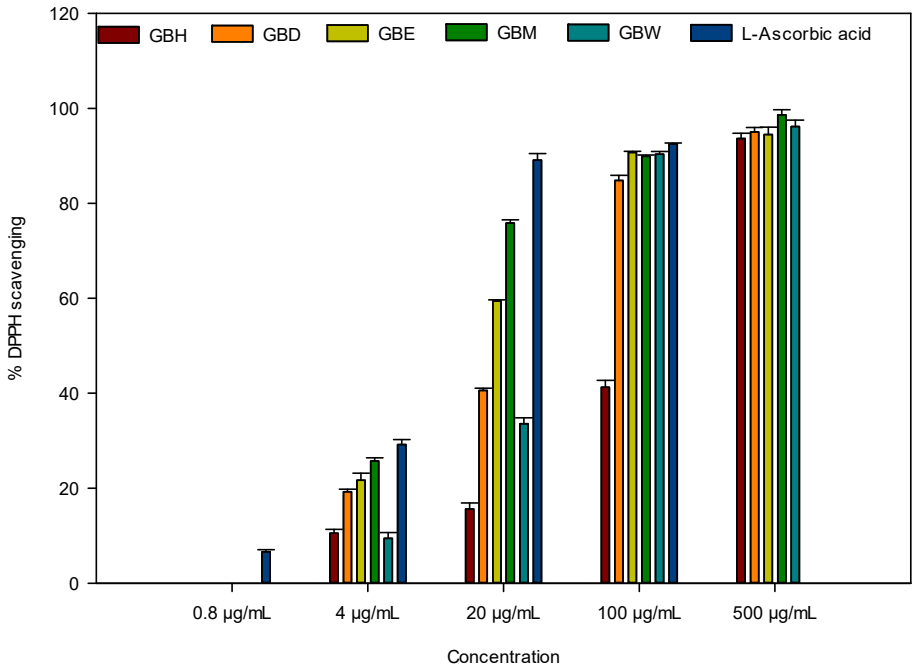


Fig 4. % DPPH scavenging efficiency of *Grewia bulot* leaf extracts

antioxidant [31] and indicates the presence of free-radical-scavenging active metabolites, such as 2-methoxy-4-vinylphenol [32], loliolide [33], phytol [34], neophytadiene [35], squalene [36], and α-tocopherol [37]. Previously, the methanol extract of *G. villosa* showed the weakest DPPH scavenging effect at 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL, comparable to that of standard vitamin E [38], while the methanol and acetone extracts of *G. optiva* leaves did not possess antioxidant activity [39]. In another study, the antioxidant activities of the syrup, jam, and seed of *G. tenax* were evaluated, with the seed extract containing the highest antioxidant content [40]. Further, the antioxidant potential was highest in *G. tenax* (85.49 ± 2.68 µg/mL) and lowest in *G. tiliifolia* (76.11 ± 1.77 µg/mL) and *G. asiatica* (82.5 ± 5.66 µg/mL) [41]. As determined via DPPH assays, the methanol extract of *G. sapida* showed antioxidant activity, with an IC_{50} value of 257.666 ± 2.516 µg/mL [42]. In another study, the crude chloroform and methanol stem bark extracts of *G. lasiocarpa* showed the highest inhibition with IC_{50} of 92.94 and 75.19 µg/mL for the FRAP and DPPH assays, respectively, in terms of antioxidant activity [29]. Further, an evaluation of the methanol extract of *G. asiatica* investigated the significant antioxidant activity of its fruits [43], and the petroleum ether fraction of *G. abutilifolia* leaf had the highest activity (IC_{50} = 3.82 ± 0.055 µg/mL) in a DPPH scavenging assay [44]. Comparing the DPPH radical scavenging activity of *G. bulot* with that of other *Grewia* species reported in the literature implies that in most cases, *G. bulot* exhibits stronger activity; therefore, it can be used as a potential source of ethnic medicinal plants to develop new forms of antioxidant therapy.

■ CONCLUSION

The current study provides a comprehensive analysis of the chemical compositions of Vietnamese *Grewia bulot* leaf extracts and investigates their antioxidant and anticancer activities. The main chemical classes identified in the *n*-hexane extract were fatty acid esters (31.6%), diterpenes (18.2%), and steroids (6.3%). In contrast, the dichloromethane extract contained alkanes (31.2%), fatty acids (14.6%), alkenes (10.6%), fatty acid esters (9.4%), and alcohols (6.9%). Further, the major compounds found in the *n*-hexane extract were neophytadiene (18.2%), methyl palmitate (14.4%), methyl linoleate (9.7%), and β-sitosterol (4.5%). On the other hand, the dichloromethane extract was

characterized by palmitic acid (9.8%), hexadecane (7.4%), octadecane (6.0%), 2-*tert*-butoxyethanol (5.3%), and neophytadiene (5.3%). Both extracts exhibited weak activity against four human cancer cell lines (MCF-7, Hep-G2, SK-LU-1, and KB), with IC_{50} values ranging from 90.60 ± 3.49 to 98.27 ± 2.77 μg/mL. Additionally, all five crude extracts displayed significant antioxidant potential, with the methanol extract showing the highest activity (SC_{50} = 9.39 ± 0.90 μg/mL). These findings suggest the potential application of *Grewia bulot* leaf extracts as a source of antioxidants.

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■ AUTHOR CONTRIBUTIONS

Ty Viet Pham conducted the experiment, Anh Tuan Le, Y Duy Ngo, Nhan Thi Thanh Dang, and Thang Quoc Le analyzed the data, Bao Chi Nguyen, and Duc Viet Ho wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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