GC-MS ANALYSIS ON VOLATILE COMPOUNDS, *IN VITRO* ANTIOXIDANT AND ANTI-HEPG2 ACTIVITIES OF THE ETHANOLIC EXTRACT AND ITS FRACTIONS FROM WHOLE PLANTS OF *Ludwigia hyssopifolia*

Huynh Anh Duy*, Tran Thi Kim Nga, Huynh Phu Vinh
Can Tho University

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ABSTRACT

Ludwigia hyssopifolia was used in folk practices to treat jaundice diseases in many Asian countries. This study aims to evaluate the antioxidant and anti-cancerous properties of the solvent extractives. The crude ethanolic extract and its fractions (chloroform, ethyl acetate, and methanol) were prepared from the whole plants. These extracts were subjected to DPPH assay to determine the antioxidant properties. An MTT assay was performed to investigate the cytotoxic activity against HepG2 cells. The volatile compounds from the ethanolic extract were identified by GC-MS technique. Total polyphenol and total flavonoid contents were determined by the spectrophotometric method. From the ethanolic extract of the whole plant, 15 phytoconstituents were analyzed. Crocetane (19.40%), heneicosane (14.85%), and 3,5-di-tert-butylphenol (14.82%) were identified as the main phytoconstituents. The ethyl acetate and methanolic fraction had the highest total polyphenol and flavonoid contents. Ethyl acetate and methanolic fractions had the best DPPH radical scavenging activity. Ethanolic extract and chloroform fraction showed the most potential anti-HepG2 activity. *Ludwigia hyssopifolia* represents a promising resource for developing of potential antioxidant and hepatoprotective products.

PHÂN TÍCH CÁC HỢP CHẤT DỄ BAY HƠI, SÀNG LỌC TÁC DỤNG KHÁNG OXY HÓA, ỨC CHẾ TẾ BÀO HepG2 *IN VITRO* CỦA CAO ETHANOL VÀ CÁC PHÂN ĐOẠN TỪ TOÀN CÂY RAU MƯƠNG THON (*Ludwidgia hyssopifolia)*

Huỳnh Anh Duy*, Trần Thị Kim Ngà, Huỳnh Phú Vinh
Trường Đại học Cần Thơ

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TỪ KHÓA

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Toàn cây
Kháng oxy hóa
Ức chế HepG2
GC-MS

TÓM TẮT

Ludwigia hyssopifolia được sử dụng trong dân gian để điều trị bệnh vàng da ở nhiều nước châu Á. Nghiên cứu nhằm đánh giá đặc tính kháng oxy hóa và ức chế dòng tế bào HepG2 của cao chiết. Cao ethanol và phân đoạn chloroform, ethyl acetat và methanol được chuẩn bị. Mẫu được khảo sát đặc tính kháng oxy hóa trên mô hình DPPH. Thử nghiệm MTT được thực hiện để nghiên cứu hoạt tính ức chế HepG2. Các hợp chất dễ bay hơi từ cao ethanol được xác định bằng GC-MS. Hàm lượng polyphenol và flavonoid toàn phần được xác định bằng phương pháp quang phổ. Cao ethanol ghi nhận 15 cấu tử, với crocetane, heneicosane và 3,5-di-tert-butylphenol là các thành phần chính. Phân đoạn ethyl acetat và methanol có hàm lượng polyphenol và flavonoid cao nhất cũng như có hoạt tính kháng oxy hóa tốt nhất. Cao ethanol toàn phần và phân đoạn chloroform cho hoạt tính ức chế HepG2 tiềm năng nhất. Do đó, *L. hyssopifolia* có tiềm năng để phát triển các sản phẩm chống oxy hóa và bảo vệ gan.

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* Corresponding author. *Email: haduy@ctu.edu.vn*

1. Introduction

With over 800,000 deaths a year, liver cancer ranks as the fourth most common cause of death worldwide. Of all primary liver cancers, hepatocellular carcinoma accounts for over 90% [1]. Jaundice presents in 19% to 40% of patients with hepatocellular carcinoma (HCC) at the time of diagnosis and usually occurs in later stages [2]. By observing of various statistics, natural products have proven to be an important source of treatment and prevention for liver cancer, providing an overview that clarifies their effectiveness and mechanisms of action [3], [4].

Ludwigia hyssopifolia (G. Don) Exell, commonly known as the water primrose in English and "Rau mương thon" in Vietnamese, is extensively grown in many Asian nations, including Vietnam. The plant is conventionally used in India to treatment of jaundice [5]. Notably, this sign is one of the most common among the different symptoms of liver disorders. It is not a disease but a symptom of liver disease that indicates liver malfunctioning [6].

Besides, as explained by review articles, *Ludwigia hyssopifolia* contains various active phytochemicals, including flavonoids, steroids, triterpenoids, phenolics, and coumarics [7]. Remarkably, these natural subtances were demonstrated to be responsible for the therapeutic effect on jaundice [6].

Praneetha et al. (2018) investigated *in vitro* and *in vivo* hepatoprotective activity of methanolic extract of aerial parts of *Ludwigia hyssopifolia* collected in India by anti-HepG2 cells assay. HepG2 was isolated from hepatocellular carcinoma, possessing high proliferation rates, making it most popular in drug metabolism and hepatotoxicity studies [8]. Results showed that HepG2 cells treated with different concentrations of methanolic extracts (50, 100, and 200 µg/ml) exhibited a significant restoration of altered levels of hepatic enzymes and HepG2 cell growth inhibition, which was comparable to that of standard drug (silymarin) [9]. However, there are few studies on the hepatoprotective effect of this medicinal plant in Vietnam.

From the justifications above, this paper aimed to screen the *in vitro* antioxidant and anti-HepG2 cell properties of the crude ethanolic extract and its fractions from *Ludwigia hyssopifolia* grown in the Mekong Delta, Vietnam. In addition, the volatile organic compound profile of total ethanolic extract was investigated using GC-MS analysis. Furthermore, based on indigenous knowledge, the whole plant was the part most commonly used in Vietnamese folk remedies, possibly because of its convenience in harvesting. Hence, it was chosen as experimental material for our study.

2. Materials and Methods

2.1. Plant samples

Figure 1. *Whole plant of Ludwigia hyssopifolia*
(Location: Thanh Binh district, Dong Thap province, Vietnam (10.56573° N, 105.54435° E))

Whole plants of *Ludwigia hyssopifolia* were collected from Thanh Binh district, Dong Thap province, Vietnam (10.56573° N, 105.54435° E), in December 2022 (Figure 1). The plant material was botanically identified by Msc. Phung Thi Hang, Department of Biology Education, School of Education, Can Tho University, combined with plant DNA barcoding identification results from previous research [10]. These samples were cut into small pieces and air dried under shade. Then, they were powdered with an electric blender.

2.2. Methods

2.2.1. Extraction procedure

Approximately 100 g of plant materials of *Ludwigia hyssopifolia* powder was added to 800 mL ethanol and soaked for 24 hours. The residue was re-extracted under the same condition two more times. The combined ethanol specimen was evaporated to dryness using a vacuum rotary evaporator. The crude ethanolic sample was coded as TE50. In addition, the total ethanolic extract was continuously mixed with celite 545 and successfully solid-liquid extracted with chloroform, ethyl acetate, and methanol. The solvents were removed from the extracts under vacuum to get corresponding fractions. The chloroform, ethyl acetate, and methanol fractions were coded as CE50, AE50, and ME50, respectively. These ethanolic extracts and fractions obtained were subject further analysis.

2.2. Gas chromatography and Mass spectroscopy (GC-MS) analysis

GC-MS analysis of ethanolic extract of *Ludwigia hyssopifolia* was performed in Can Tho Technical Center of Standards Metrology and Quality (CATECH). The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 μm film. The carrier gas used is Helium with at flow of 1.0 ml/min. The sample volume is 2.0 μl. The injector was operated at 250 °C and the oven temperature was programmed as follows: 50 °C for 02 min, then gradually increased at 5 °C/min to 100 °C, 10 °C/min to 150 °C, 25 °C/min to 250 °C. The identification of components was based on Wiley and NIST libraries and comparing of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST and Wiley) attached to the GC-MS instrument and the results obtained have been tabulated.

2.2.3. Phytochemical analysis

Total Polyphenol Content Assessment: The total phenolic content was measured according to the method of Singleton et al. (1999). Slowly, 0.5 mL of sample was added to 4.5 mL of distilled water and was mixed with 0.2 mL of the Folin–Ciocalteu phenol reagent and 0.5 mL saturated solution of Na_2CO_3. Finally, 4.3 mL of distilled water was added to the solution. The reaction mixtures were incubated for 60 min in the dark at room temperature and then, the absorbances were measured at 765 nm. Total phenolic content was expressed as mg of Gallic acid equivalents (GAE) per gram of dry sample (mg GAE/g).

Total flavonoid Content Assessment: The total flavonoid content was measured by a colorimetric assay of Zhishen et al. (1999). One hundred microliters of extract was added to 4 ml of distilled water. Then, 0.3 ml 5% sodium nitrite was added. After 5 min, 0.3 ml of 10% aluminum chloride was added. In 6 min, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the mixture was diluted by adding of 3.3 ml of distilled water and mixed thoroughly. The absorbance was determined at 510 nm versus a blank. Quercetin was used as a standard for the calibration curve. The total flavonoid content of the extract was expressed as mg quercetin equivalents per gram of sample (mg/g).

2.2.5. Antioxidant activity

Antioxidant of the crude ethanolic extract and its fractions of *Ludwigia hyssopifolia* was tested using a 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay as described previously. The samples were dissolved in MeOH, and the solutions were dispensed into wells of a 96-well microplate with an appropriate volume. Briefly, 40 µL of DPPH solution (Tokyo Chemical Industry, Japan, 1000 µg/mL, in methanol) was incubated with varying concentrations of the different extracts. The final concentrations of tested samples in the mixtures ranged from 256 to 8 µg/mL. The reaction mixture was shaken well and incubated for 30 min in the dark at ambient temperature. The absorbance of the resulting solutions was measured at λ = 520 nm. Pure MeOH was used as a negative control and milk thistle extract (53,8% silymarin, National Institute of Drug Quality Control, Vietnam) was used as a positive control from the three replicates. All data on antioxidant activity are the average of triplicate experiments. The radical scavenging activity of the extracts was expressed as IC_{50} (the concentration of the sample required to inhibit 50% of the DPPH concentration). The IC_{50} values were calculated using a linear regression of plots, where the abscissa represented the concentration of tested plant extracts and the ordinate the average percentage of scavenging capacity.

2.2.6. Anti-Hepatocellular Carcinoma HepG2 Activity

The MTT assay assessed the anti-HepG2 cell activity of tested samples. Briefly, 190 µl HepG2 cells (1-3 × 10^3 cells/well) were maintained in 96 well‑culture plates in the presence of 10 µl of LHME at the various concentrations for 72 h in a standard condition. After incubation, 10 µL MTT reagent (5 mg/mL) was added, and cell cultures were incubated for 4 hours. A quantity of 100 µL DMSO 100% was added to dissolve the resultant formazan crystals after the medium was removed. The wells' absorbance (Abs) was measured was measured at in a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at a wavelength of 540 nm. The experiment was repeated 3 times. Wells without cells serve as a negative control, and their absorbance has to be subtracted from the other results. Untreated cells are the positive control. The percentage of growth inhibition (%) of HepG2 cells is calculated as the percentage of viability of the untreated cells as described in equation (1).

$$\%\text{Inhibition} = (Abs_{control\ (+)} - Abs_{test})/(Abs_{control\ (+)} - Abs_{control\ (-)}) \times 100\% \quad (1)$$

The IC_{50} value (50% of cytotoxicity inhibition) was determined through the %Inhibition of growth and Rawdata software as described in equation (1).

$$IC_{50} = High_{Conc} - \frac{(High_{Inh\%} - 50) \times (High_{Conc} - Low_{Conc})}{High_{Inh\%} - Low_{Inh\%}} \quad (2)$$

$High_{Conc}$/Low_{Conc}: Tested samples at high concentration/Tested samples at low concentration.

$High_{Inh\%}$/$Low_{Inh\%}$: %Inhibition at high concentration/%Inhibition at low concentration.

3. Results and Discussion

3.1. GC-MS analysis of the whole plant ethanolic extract of Ludwigia hyssopifolia

Medicinal plants have contributed many ingredients to fight against various pathological conditions. Hence, the present study used GC-MS analysis to find the bioactive compounds in the whole plant ethanolic extract of *Ludwigia hyssopifolia*. The active principles with their retention time (RT), class, molecular formula, and peak area % were displayed in Table 1 and Fig. 2, which showed the presence of 15 phytoconstituents in the ethanolic extract of whole plant of *Ludwigia hyssopifolia*. The chemical profiling of ethanolic whole plant extracts found crocetane (19.40%), heneicosane (14.85%), and 3,5-di-tert-butylphenol (14.82%) as the main compounds in this organ while some notable compounds with small amount such as dihydroactinidiolide (6.62%), borneol (4.48%), D(+)camphor (3.79%), isolongifolene (3.42%) along with bioactive alkane substances pentadecane (3.37%), tetradecane (3.20%), and heptadecane (1.72%). In general, non-oxygenated hydrocarbons accounted for the highest percentage (56.99%), followed by aromatic compounds (22.65%), and terpenoids (18.31%).

Table 1. *GC-MS spectral analysis of whole plant ethanolic extract of Ludwigia hyssopifolia*

No. Peak	RT (min)	Name of compounds	Class	Molecular formula	Peak Area %
1	8.992	3-Ethyl-3-methylheptane	Non-oxygenated hydrocarbons	$C_{10}H_{22}$	5.00
2	10.832	D(+)Camphor	Bicyclic monoterpene	$C_{10}H_{16}O$	3.79
3	11.239	Borneol	Bicyclic monoterpene	$C_{10}H_{18}O$	4.48
4	11.538	Tetradecane	Non-oxygenated hydrocarbons	$C_{14}H_{30}$	3.20
5	12.525	Heptadecane	Non-oxygenated hydrocarbons	$C_{17}H_{36}$	1.72
6	12.730	4,6-Dimethyldodecane	Non-oxygenated hydrocarbons	$C_{14}H_{30}$	6.70
7	13.445	2,6,11-Trimethyldodecane	Non-oxygenated hydrocarbons	$C_{15}H_{32}$	2.75
8	14.626	(2,3-Dimethyldecyl)benzene	Aromatic compounds	$C_{10}H_{12}$	7.83
9	15.590	Pentadecane	Non-oxygenated hydrocarbons	$C_{15}H_{32}$	3.37
10	15.882	Isolongifolene	Tricyclic sesquiterpene	$C_{15}H_{24}$	3.42
11	16.013	Heneicosane	Non-oxygenated hydrocarbons	$C_{21}H_{44}$	14.85
12	16.368	3,5-Di-tert-butylphenol	Aromatic compounds	$C_{14}H_{22}O$	14.82
13	16.535	No determined	-	-	2.05
14	16.808	Crocetane	Non-oxygenated hydrocarbons	$C_{20}H_{42}$	19.40
15	17.145	Dihydroactinidiolide	Sesquiterpene lactones	$C_{11}H_{16}O_2$	6.62
			Total		100

Crocetane (2,6,11,15-tetramethylhexadecane) is an irregular tail-to-tail linked isoprenoid hydrocarbon, and has been detected in modern sediments and geological records as a biomarker, often associated with anaerobic methane oxidation. Besides, it is one of the important biomarkers for hypersaline depositional environments [11]. Next, heneicosane was one of the important components of *Carthamus tinctorius* flowers essential oil [12], and was identified as a novel microbicidal bioactive alkane from *Plumbago zeylanica*. This compound exhibited excellent antimicrobial activity against *Streptococcus pneumoniae* and *Aspergillus fumigatus* at 10 μg/ml concentrations [13] and possessed antimicrobial, anticancerous, and antioxidant activities [14]. Meanwhile, 3,5-Di-tert-butylphenol (3,5-DTB) was identified as the major constituent in the methanolic extract of *Pleurotus florida* and exhibited combined anti-biofilm and fungicidal activity against *Candida* species [15].

Dihydroactinidiolide, a degradation product of carotenoids, that has been found to be responsible for the aroma of black tea and tobacco. This compound has antioxidant activity, antibacterial activity, anticancer activity and neuroprotective effect [16], [17]. Interestingly, it is used as a pheromone by some higher plants and insects [16]. Furthermore, borneol is a bicyclic monoterpenoid component present in the essential oils of numerous medicinal plants, such as valerian, chamomile and lavender, and has been used as an analgesic, antibacterial, and anti-inflammatory agent. Borneol also has a strong anticancer potential against different cancers such as hepatocellular carcinoma, neuroblastoma, glioma, esophageal squamous cell carcinoma, and ovarian and lung cancer [18]. Recent evidence has led to extensive research in establishing borneol as a permeation enhancer across the blood-brain barrier. Owing to these mechanisms of altering membrane properties, borneol has also shown promising potential to enhance the distribution of drugs in brain tissues [19]. For example, the statistics demonstrated that natural

borneol acted as an adjuvant agent to promote cellular uptake of piperlongumine to improve its anti-glioma efficacy [20], or it can be further developed as a chemical sensitizer of selenocystine in human cancer treatment [21]. D(+)Camphor is also a major essential oil component of many aromatic plant species. It exhibits several biological properties such as insecticidal, antimicrobial, antiviral, anticoccidial, anti-nociceptive, anticancer, and antitussive activities, and its use as a skin penetration enhancer [22], [23]. At the same time, isolongifolene is a tricyclic sesquiterpene isolated from *Murraya koenigii*, with antioxidant, anti-inflammatory, anti-apoptosis, anticancer, and neuroprotective properties [24], [25].

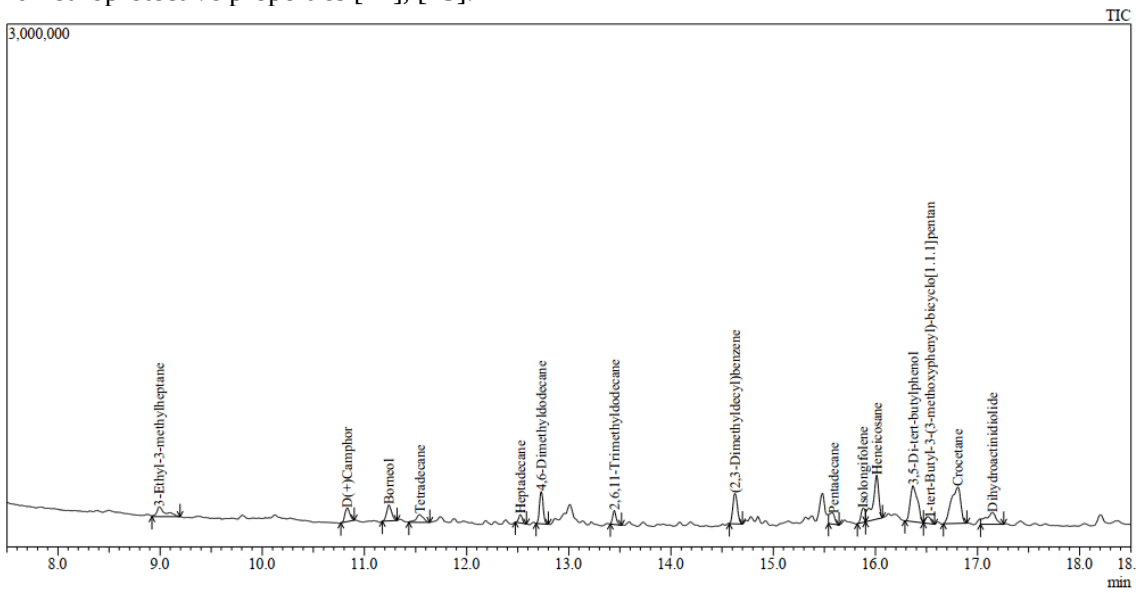

Figure 2. *GC-MS chromatogram for whole plant ethanolic extract of Ludwigia hyssopifolia*

Pentadecane is a straight-chain alkane with 15 carbon atoms. It is a component of volatile oils isolated from *Scandix balansae*, and it shows antimicrobial activity against *Leishmania infantum* parasites in *in vitro* culture [26]. At the same time, tetradecane has antimicrobial, antifungal, and antitumor properties [27]. Finally, heptadecane is a straight-chain alkane with 17 carbon atoms and is a component of essential oils from plants like *Opuntia littoralis* and *Annona squamosa*. Moreover, heptadecane is also major component of *Spirulina platensis*. It has been shown that the strong antioxidative effects have therapeutic benefit in a rat renal disease model, and inhibit the proliferation of human liver cancer cells [28], [29].

3.2. Phytochemical analysis

The total phenolic and flavonoid contents of the crude ethanolic extract and its fractions from *Ludwigia hyssopifolia* whole plant were evaluated. The results have been revealed in Table 2.

Table 2. *The total phenolic and flavonoid contents of ethanolic extract and its fractions of Ludwigia hyssopifolia whole plant*

Test samples	Sample codes	Total polyphenol content (mg GAE/g)	Total flavonoid content (mg QE/g)
Crude ethanolic extract	TE50	28.09±5.44	5.95±0.77
Chloroform fraction	CE50	44.39±1.08	9.02±1.54
Ethyl acetate fraction	AE50	243.27±17.24	82.3±5.45
Methanolic fraction	ME50	237.81±10.58	86.9±9.00

In terms of total phenolic content, values of ethyl acetate fraction (243.27±17.24 mg GAE/g), methanolic fraction (237.81±10.58 mg GAE/g) was higher than that of chloroform fraction (44.39±1.08 mg GAE/g) and crude ethanolic extract (28.09±5.44mg GAE/g). Meanwhile, the

total flavonoid content of methanolic fraction (86.9±9.00 mg QE/g) was higher than ethyl acetate fraction (82.3±5.45 mg (QE/g), chloroform fraction (9.02±1.54 mg QE/g) and crude ethanolic extract (5.95±0.77 mg QE/g). Among tested samples, ethyl acetate fraction had the highest total polyphenol content and methanolic fraction showed most significant figure in total flavonoid content. In addition, *L. hyssopifolia* total ethanolic extract displayed lower phenolic and flavonoid contents than the other samples.

3.3. Antioxidant activity

Antioxidant activity of solvent extracts was evaluated by DPPH assays. Table 3 shows the free radical scavenging activity of the ethanolic extracts and its fractions of *L. hyssopifolia* and milk thistle extract (standard drug). Among the extractives, ethyl acetate fraction (AE50) possessed the highest activity with IC_{50} being 9.75 µg/mL. Meanwhile, the IC_{50} of methanolic fraction (ME50), total ethanolic extract (TE50), and chloroform fraction (CE50) were 19.51, 69.75, and 88.37 µg/mL, respectively. The IC_{50} of milk thistle extract (SM01) (standard) was 27.44 µg/mL. The DPPH free radical scavenging activity of different samples and milk thistle extract was in the following order: AE50 > ME50 > SM01 > TE50 > CE50. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer. This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. Interestingly, our results revealed that ethyl acetate and methanolic fractions of *L. hyssopifolia* had a greater free radical scavenging activity when compared with standard milk thistle extract. The previous study demonstrated that milk thistle extract had good DPPH free radical scavenging activity and might prevent lipid peroxidation [30]. Besides, total polyphenol, flavonoid content and radical scavenging antioxidant activity are highly correlated based on multiple statistics [31], [32]. Phenolic compounds are good electron donors because their hydroxyl groups can directly contribute to antioxidant action [33]. As a result, ethyl acetate and methanolic fractions of *L. hyssopifolia* whole plant may be used as potentially rich sources of antioxidants.

Table 3. *IC_{50} values (µg/mL) of extractives on DPPH assay*

Test samples	Sample codes	Regression equation	IC_{50} (µg/mL)
Total ethanolic extract	TE50	y = 0.499x + 15,197 (R^2 = 0.9947)	69.75
Chloroform fraction	CE50	y = 0.4568x + 9,6345 (R^2 = 0.9944)	88.37
Ethyl acetate fraction	AE50	y = 6.5593x – 13,977 (R^2 = 0.9907)	9.75
Methanolic fraction	ME50	y = 2.7338x – 3,3275 (R^2 = 0.9843)	19.51
Milk thistle extract	SM01	y = 0.4167x + 38,656 (R^2 = 0.9616)	27.44

3.4. Anti-Hepatocellular Carcinoma HepG2 Activity

Four extracts that included total ethanol extracts and solvent fractions were assessed for anti-cancer activity using HepG2 cells *in vitro* (Table 4). The result demonstrated that the ethanolic crude whole plant extract (TE50) and chloroform fraction (CE50) of *L. hyssopifolia* showed greater potential anticancer activity (IC_{50} of 140.82±1.67 and 163.84±3.62 µg/mL, respectively) than all samples. Notable, IC_{50} values of two fractions were lower than that of milk thistle extract (SM01) (IC_{50} = 179.2 ± 4.18 µg/mL). Milk thistle (*Silybum marianum*) is a medicinal plant from the Asteraceae family. Silymarin is the major constituent of milk thistle extract and is a mixture of some flavonolignans, which is the most active component of silymarin. It is most commonly known for its chemopreventive effects against cancers [34], [35]. Meanwhile, ethyl actetate (AE50) and a methanolic fraction (ME50) displayed almost no anti-HepG2 properties (IC_{50} > 256 µg/mL). Therefore, these preliminary data demonstrated the potential of the ethanolic extract (TE50) and chloroform fraction (CE50) of *L. hyssopifolia* whole plant to anti-HepG2 effects.

Notably, the ethanolic extract had the highest activity among all extractives, which suggested a synergistic effect on cytotoxic activity against HepG2 cells of medicinal herbs.

Table 4 . *IC_{50} values (µg/mL) of test samples of cytotoxic assay on HepG2 cells*

Sample codes	Conc. (µg/ml)	Growth inhibition (%)	IC_{50} (µg/ml)	Sample codes	Conc. (µg/ml)	Growth inhibition (%)	IC_{50} (µg/ml)
TE50	256	89	140.82± 1.67	**AE50**	256	16	>256
	64	24			64	11	
	16	20			16	9	
	4	11			4	5	
CE50	256	86	163.84± 3.62	**ME50**	256	22	>256
	64	11			64	15	
	16	6			16	7	
	4	5			4	0	
Milk thistle extract	256	75.5	179.2 ± 4.18				
	64	11					
	16	7					
	4	3					

4. Conclusions

The identification of bioactive compounds in the ethanolic extract of *Ludwigia hyssopifolia* whole plant was done by GC-MS analysis, which showed the presence of 15 compounds. Among the identified compounds, crocetane, heneicosane, and 3,5-di-tert-butylphenol were major phytoconstituents. Among tested samples, ethyl acetate fraction had the highest total polyphenol content and methanolic fraction showed the greatest figure in total flavonoid content. In addition, *L. hyssopifolia* total ethanolic extract displayed the lower phenolic and flavonoid contents than the other samples. The ethyl acetate and methanolic fractions of *L. hyssopifolia* showed the highest free radical scavenging activities in the DPPH assay. In contrast, the crude ethanolic extract and chloroform fraction had the best potential anti-HepG2 properties.

REFERENCES

[1] J. H. Oh and D. W. Jun, "The latest global burden of liver cancer: A past and present threat," *Clin Mol Hepatol*, vol. 29, no. 2, pp. 355-357, 2023.

[2] L. X. Qin and Z. Y. Tang, "Hepatocellular carcinoma with obstructive jaundice: diagnosis, treatment and prognosis," *World J Gastroenterol*, vol. 9, no. 3, pp. 385-391, 2003.

[3] A. K. Singh, S. V. Singh, R. Kumar, S. Kumar, S. Senapati, and A. K. Pandey, "Current therapeutic modalities and chemopreventive role of natural products in liver cancer: Progress and promise," *World J Hepatol*, vol. 15, no. 1, pp. 1-18, 2023.

[4] D. B. Kim, D. K. Lee, C. Cheon, R. Ribeiro, and B. Kim, "Natural Products for Liver Cancer Treatment: From Traditional Medicine to Modern Drug Discovery," *Nutrients*, vol. 14, no. 20, p. 4252, 2022.

[5] V. Deepak, S. Arumugam, C. Amritha, P. Prajitha, and H. Faslu, "Phytopharmacological activities of Ludwigia hyssopifolia (g. Don) exell: a review," *Asian Journal of Research in Chemistry and Pharmaceutical Sciences*, vol. 7, no. 2, pp. 781-789, 2019.

[6] V. Janghel, P. Patel, and S. S. Chandel, "Plants used for the treatment of icterus (jaundice) in Central India: A review," *Annals of Hepatology*, vol. 18, no. 5, pp. 658-672, 2019.

[7] Q. D. Tran, T. S. Khuu, T. T. G. Le, M. T. A. Nguyen, T. N. G. Ngo, and T. T. T. Nguyen, "A review on chemical constituents of Ludwigia genus in Vietnam," *Cantho Journal of Medicine and Pharmacy*, vol. 65, pp. 234-243, 2023.

[8] M. T. Donato, L. Tolosa, and M. J. Gómez-Lechón, "Culture and Functional Characterization of Human Hepatoma HepG2 Cells," *Methods in molecular biology*, vol. 1250, pp. 77-93, 2015.

[9] P. Praneetha, Y. N. Reddy, and B. R. Kumar, "In vitro and In vivo hepatoprotective studies on methanolic extract of aerial parts of Ludwigia hyssopifolia G. Don Exell," *Pharmacognosy Magazine*, vol. 14, no. 59s, pp. s546-s553, 2018.

[10] A. D. Huynh, P. V. Huynh, V. H. Nguyen, and T. K. N. Tran, "In vitro anti-oxidant, anti-HepG2 properties of aqueous extracts from various parts of Ludwidgia hyssopifolia," *TNU Journal of Science and Technology*, vol. 229, no. 02, pp. 148-155, 2024.

[11] K. Huang and D. Armstrong, "GC-MS analysis of crocetane, phytane and some of their stereoisomers using cyclodextrin-based stationary phases," *Organic Geochemistry*, vol. 40, pp. 283-286, 2009.

[12] J. Asgarpanah and N. Kazemivash, "Phytochemistry, pharmacology and medicinal properties of Carthamus tinctorius L.," *Chin J Integr Med*, vol. 19, no. 2, pp. 153-159, 2013.

[13] V. Vanitha, S. Vijayakumar, M. Nilavukkarasi, V. N. Punitha, E. Vidhya, and P. K. Praseetha, "Heneicosane—A novel microbicidal bioactive alkane identified from Plumbago zeylanica L.," *Industrial Crops and Products*, vol. 154, 2020, Art. no. 112748.

[14] M. Sharma, S. Bharti, A. Goswami, and S. Mallubhotla, "Diversity, Antimicrobial, Antioxidant, and Anticancer Activity of Culturable Fungal Endophyte Communities in Cordia dichotoma," *Molecules*, vol. 28, no. 19, p. 6926, 2023.

[15] J. Rathna, D. Bakkiyaraj, and S. K. Pandian, "Anti-biofilm mechanisms of 3,5-di-tert-butylphenol against clinically relevant fungal pathogens," *Biofouling*, vol. 32, no. 9, pp. 979-993, 2016.

[16] M. Das *et al.*, "Dihydroactinidiolide, a natural product against Aβ(25-35) induced toxicity in Neuro2a cells: Synthesis, in silico and in vitro studies," *Bioorg Chem*, vol. 81, pp. 340-349, 2018.

[17] M. Amal *et al.*, "Chemical constituents and biological activities of different solvent extracts of Prosopis farcta growing in Egypt," *Journal of Pharmacognosy and Phytotherapy*, vol. 9, pp. 67-76, 2017.

[18] I. Naureen *et al.*, "Borneol as Adjuvant Chemotherapy: A New Way for the Development of Novel Chemotherapeutic," *Haya: The Saudi Journal of Life Sciences*, vol. 7, no. 4, pp. 128-141, 2022.

[19] M. Kulkarni, N. Sawant, A. Kolapkar, A. Huprikar, and N. Desai, "Borneol: a Promising Monoterpenoid in Enhancing Drug Delivery Across Various Physiological Barriers," *AAPS PharmSciTech*, vol. 22, no. 4, p. 145, 2021.

[20] M. Wang *et al.*, "Natural borneol serves as an adjuvant agent to promote the cellular uptake of piperlongumine for improving its antiglioma efficacy", *European Journal of Pharmaceutical Sciences*, vol. 181, 2023, Art. no. 106347.

[21] J. Su *et al.*, "Natural borneol, a monoterpenoid compound, potentiates selenocystine-induced apoptosis in human hepatocellular carcinoma cells by enhancement of cellular uptake and activation of ROS-mediated DNA damage," *PLoS One*, vol. 8, no. 5, 2013, Art. no. e63502.

[22] W. Chen, I. Vermaak, and A. Viljoen, "Camphor--a fumigant during the Black Death and a coveted fragrant wood in ancient Egypt and Babylon--a review," *Molecules*, vol. 18, no. 5, pp. 5434-5454, 2013.

[23] H. Singh *et al.*, "Camphor and Menthol as Anticancer Agents: Synthesis, Structure-Activity Relationship and Interaction with Cancer Cell Lines," *Anticancer Agents Med Chem*, vol. 23, no. 6, pp. 614-623, 2023.

[24] J. Li *et al.*, "Isolongifolene alleviates liver ischemia/reperfusion injury by regulating AMPK-PGC1α signaling pathway-mediated inflammation, apoptosis, and oxidative stress," *International Immunopharmacology*, vol. 113, 2022, Art. no. 109185.

[25] K. Rangasamy and E. Namasivayam, "In vitro Antioxidant and Free Radical Scavenging Activity of Isolongifolene," *Asian Journal of Biological Sciences*, vol. 5, pp. 1-11, 2014.

[26] F. Bruno *et al.*, "Cytotoxic Screening and In Vitro Evaluation of Pentadecane Against Leishmania infantum Promastigotes and Amastigotes," *J Parasitol*, vol. 101, no. 6, pp. 701-705, 2015.

[27] Y. Li, H. Qiao, R. Zhang, W. Zhang, and P. Wen, "Microbial Diversity and Volatile Flavor Compounds in Tibetan Flavor Daqu," *Foods*, vol. 12, no. 2, p. 324, 2023.

[28] L. C. Wu, J. A. Ho, M. C. Shieh, and I. W. Lu, "Antioxidant and antiproliferative activities of Spirulina and Chlorella water extracts," *J Agric Food Chem*, vol. 53, no. 10, pp. 4207-4212, 2005.

[29] D. H. Kim *et al.*, "Molecular study of dietary heptadecane for the anti-inflammatory modulation of NF-kB in the aged kidney," *PLoS One*, vol. 8, no. 3, 2013, Art. no. e59316.

[30] A. Serçe *et al.*, "Assessment of the Antioxidant Activity of Silybum marianum Seed Extract and Its Protective Effect against DNA Oxidation, Protein Damage and Lipid Peroxidation," *Food Technol Biotechnol*, vol. 54, no. 4, pp. 455-461, 2016.

[31] S. Martínez, C. Fuentes, and J. Carballo, "Antioxidant Activity, Total Phenolic Content and Total Flavonoid Content in Sweet Chestnut (Castanea sativa Mill.) Cultivars Grown in Northwest Spain under Different Environmental Conditions," *Foods*, vol. 11, no. 21, 2022, Art. no. 3519.

[32] S. Aryal, M. K. Baniya, K. Danekhu, P. Kunwar, R. Gurung, and N. Koirala, "Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal," *Plants (Basel)*, vol. 8, no. 4, p. 96, 2019.

[33] E. Bendary, R. Francis, H. Ali, M. Sarwat, and S. El Hady, "Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds," *Annals of Agricultural Sciences*, vol. 58, no. 2, pp. 173-181, 2013.

[34] V. Soleimani, P. S. Delghandi, S. A. Moallem, and G. Karimi, "Safety and toxicity of silymarin, the major constituent of milk thistle extract: An updated review," *Phytother Res*, vol. 33, no. 6, pp. 1627-1638, 2019.

[35] S. A. Emadi, M. Ghasemzadeh Rahbardar, S. Mehri, and H. Hosseinzadeh, "A review of therapeutic potentials of milk thistle (Silybum marianum L.) and its main constituent, silymarin, on cancer, and their related patents," *Iran J Basic Med Sci*, vol. 25, no. 10, pp. 1166-1176, 2022.