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Phytochemical, Antioxidant and Anti-Cancer Properties of *Urtica dioica*

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Abstract

Stinging Nettle (*Urtica dioica*) is a distinct herbaceous perennial flowering plant characterized by its peculiar stinging hairs. The objective of this research is to extract phenolic compounds and fatty acids from Nettle leaves and investigate their antioxidant and anticancer properties using column chromatography (CC), thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC). The antioxidant activity of the phenolic compounds was determined using the DPPH test. The results revealed significant antioxidant effects, with the highest percentage observed in the compounds obtained from Part II reaching 75.58% at a concentration of 200 µg/ml. Similarly, the phenolic compounds identified in Part I exhibited an antioxidant effect of 74.07% at the same concentration. In comparison, the fatty acids displayed lower efficacy, showing an antioxidant effect of 49.77% at the aforementioned concentration. Notably, the standard antioxidant, ascorbic acid, exhibited superior performance, achieving an antioxidant effect of 80.36% at the same concentration. The MTT cytotoxicity test results conducted on the HepG2 hepatocellular carcinoma cell line and the WRL68 normal cell line revealed that the phenolic compounds exhibited toxicity specifically towards the HepG2 cancer line. In Part I, it was observed that coumaric acid displayed the highest inhibition rate, reaching 72.3% at a concentration of 400 µg/ml, while it did not exhibit a significantly toxic effect on the WRL68 normal cells. Subsequently, in Part II, the phenolic compounds demonstrated a percentage of inhibition of 68.21%, which was the lowest level of inhibition observed on the normal WRL68 cells. Conversely, the fatty acids exhibited a lower level of efficacy, with 60.65% of the cells being prevented from proliferating at the aforementioned concentration, while the normal WRL68 cells did not experience such rapid cell death

Keywords: *Urtica dioica*, fatty acids. phenolics. Antioxidant. anticancer

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1. Introduction

Urtica dioica, commonly known as Stinging nettle, is the predominant species within the Urticaceae family and is widely recognized as one of the most extensively researched medicinal plants worldwide. This herbaceous perennial plant thrives in a variety of tropical and temperate wasteland regions across the globe, displaying remarkable adaptability to diverse environments [1,2]. The plant derives its name from the Latin verb "urere," meaning "to burn," which aptly describes the sensation caused by its stinging hairs. The species dioica, commonly encountered, refers to its tendency to bear either female or male flowers. The leaves of Urtica dioica are characterized by their oval shape, long petioles, elongated form, and toothed margins. The plant's flowers are dioecious, and its fruit manifests as small, oval-shaped achene with a greenish-yellow hue[3,4].The medicinal properties of Urtica dioica, commonly known as stinging nettle, are associated with various beneficial effects on the body. These include its ability to reduce inflammation and asthma symptoms, as well as its astringent, depurative, galactogogue, diuretic, nutritive, and stimulating properties [5,6,7]. The extract obtained from the powdered leaves of this plant has been traditionally utilized as an anti-hemorrhagic agent, effectively decreasing excessive menstrual flow and nosebleeds. The roots of Urtica dioica are employed in the treatment of benign prostate hyperplasia, while the herbaceous parts are used to address urinary tract disorders and rheumatic conditions. Additionally, fresh freeze-dried nettle leaves are commonly employed in the management of allergies [8,9]. Numerous studies have additionally documented the analgesic properties of this substance, highlighting its ability to alleviate pain. Furthermore, its effectiveness as an anti-aggregating agent has been recognized, along with its positive impact on cardiovascular function and smooth-muscle activity, making it a valuable hypotensive agent [10,12,13]. In fact, the applications of this plant extend beyond medicinal uses, finding relevance in diverse industries such as dye production, veterinary medicine, textile manufacturing, and even in cosmeceutical formulations for treating hair loss and combating dandruff.

Additionally, it is employed in culinary practices to enhance the flavor and preparation of various common dishes [14,15]. *Urtica dioica*, contains cyclic organic compounds that include steroids, forming an integral part of

its volatile oils. Additionally, it possesses various phenolic compounds, which are well-known aromatic compounds with relatively low molecular weights. These compounds consist of benzene rings connected to one or more hydroxyl groups and are frequently found in conjunction with sugars, known as glycosides. The activity of phenols in the form of glycosides is influenced by the number and position of hydroxyl groups they possess. These compounds are categorized into different groups based on the number of carbon atoms present in their chemical structure [16,17]. Natural phenolic compounds and their derivatives extracted from plants have demonstrated their potent antioxidant and anti-cancer properties. These compounds effectively combat oxidative free radicals by scavenging them. An imbalance between oxidants and antioxidants poses a significant health concern. This imbalance arises when there is an insufficient number of antioxidants or an excessive presence of oxidative free radicals within the body [18,19]. The destructive nature of these radicals leads to severe damage to normal cell components, impairing their functionality. Consequently, a rise in the number of free radicals in the body contributes to the onset of various ailments such as degenerative diseases, heart diseases, aging, neurological disorders, cancer, and other related conditions [20,21].

Urtica dioica leaves are abundant in essential fatty acids, making them a valuable and substantial source. The pursuit of fatty acid sources has gained significant significance in recent times because of their crucial role in regulating human metabolism. These fatty acids possess advantageous properties as antioxidants and are known to have a positive impact on cancer prevention. Therefore, the substantial presence of fatty acids within the tissues of Urtica dioica leaves holds immense significance for promoting human health[22,23].The objective of this study is to evaluate the antioxidant and anticancer activities of the phenolic compounds and fatty acid extract obtained from Urtica dioica leaves. To accomplish this, multiple chromatography methods were employed. thereby offering potential applications in antioxidant therapy and cancer treatment.

2. Materials and methods

2.1. Materials

During the flowering season in April and June 2020, select portions of the Urtica dioica plant unaffected by pathogens or physical harm were carefully collected from regions in northern Iraq. The plant had predominantly thrived in a soil composition consisting of silt and sand. In order to maintain cleanliness, the plant parts were handled with gloves, ensuring their preservation. Subsequently, the collected portions were securely placed within a sanitized plastic bag and transported to a laboratory. The Ministry of Agriculture personnel based in Mosul, Iraq, took charge of identifying and inspecting the plants. Following this, the plant parts were stored in a darkened environment at room temperature until they were fully dried. Once the plants had naturally desiccated, a laboratory mortar and pestle, previously cooled, were utilized to forcefully crush the dried plant parts into an exceedingly fine powder or paste. Subsequently, the pulverized material was strained through sterile cloth and stored at a temperature of 20°C, awaiting further research. The extraction, isolation, and analysis processes solely employed analytical reagents such as n-Hexan, ethyl acetate, ethanol, methanol, chloroform, vanillin, sulphuric acid, acetonitrile, phosphoric acid, p-Coumaric acid, vanillic acid, and quercetin.

2.2. Urtica extraction using a Soxhlet apparatus

The Soxhlet continuous extraction apparatus was employed for the extraction process using a sequential solvent system comprising n-Hexan, ethyl acetate, and ethanol. The extraction procedure involved the addition of 1000 ml of the respective solvent per 100 g of the plant powder sample, or 100 ml per 10 g. The extraction process lasted for 48-72 hours or until the solvent derived from the saxolith lost its color, resulting in an extract that consisted of a blend of the plant material and the utilized solvent. Subsequently, the Rotary Vacuum Evaporator (RVE) was utilized to concentrate the extracts, thereby obtaining the crude extract. The resulting extract was then stored in the refrigerator in 25 ml portions and packaged in sealed, opaque glass bottles for future use[24].

2.3. Urtica Extract Isolation and Fractionation by Column

For this method of separating, you need a stationary phase (SP), which is represented by silica gel, and a mobile phase, which is represented by the solvent. Each of these has its own properties, like polarity and the ability to hold things. In addition to the separation column, which is a glass column of different sizes filled with silica gel after being heated and used in the separation process , a number of solvents with different polarities were used in different amounts to get different parts, or "fractions" [25].

2.4. Thin-layer chromatography (TLC)

A quantity of 2 µl from each extract was placed into a thin layer chromatography (TLC) featuring an aluminum back. The TLC plates utilized in the experiment were made of glass and measured 20 cm by 20 cm. These plates were already coated with silica gel 60 F254, having a thickness of 0.2 mm, and were sourced from E. Merck/Millipore, located in Billerica, MA, USA. To prepare the TLC plate, a solution containing chloroform, ethyl acetate, and formic acid in a volumetric ratio of 10:8:2 was utilized. The developed TLC plate was subsequently examined using a UV-visible spectrophotometer that operated at wavelengths of 254 nm and 365 nm to identify fluorescent compounds. A UV spectrophotometer model Sunny UV.7804C, originating from Tokyo, Japan, was employed for this purpose. Following this step, vanillin sulphuric acid was sprayed onto the TLC plate, which was then subjected to heating in order to visualize the distinct colors of the various compounds present. For each identified spot, the Rf value was determined and compared with established standards. Once the samples had dried, their weights were measured, and the High-performance liquid chromatography (HPLC) technique was employed to confirm the identity of the phenolic compounds [26].

2.5. Analysis of fatty acids by Gas– Chromatography Mass Spectrometry (GC-MS)

A gas chromatograph (Shimadzu GC-2010) and a mass spectrometer (QP2010) were utilized to analyze the contents of the n-Hexan extract obtained from U. dioica. The sample was introduced onto a 30 m glass capillary column coated with a 0.25 m thick film and inserted into the GC-MS system (30 m 0.25 m). Helium was employed as the carrier gas, flowing consistently at a rate of 1 ml/min. Both the injector and the detector were maintained at a constant temperature of 250 °C. The GC temperature was programmed to increase from 60°C to 280°C at a rate of 15°C per minute. The injection was conducted using a 1:3 split. The entire GC process lasted for 35 minutes. The mass spectrometer was operated at 70 eV. The mass scan encompassed a range of m/z 40–1000, with a scan interval of 0.5 s, a scan speed of 1000 amu s1, and a detector voltage of 1.0 kV. To identify the compounds present, the NIST08, WILEY8, and FAME Libraries were employed as databases. A software database named Libraries was utilized to compare the mass spectrum of each unknown compound with the mass spectra of known compounds. This allowed for the determination of the names, molecular weights, and structures of the tested materials [27].

2.6. Analysis of Phenolic compounds by HPLC (High Performance Liquid Chromatography)

To confirm the presence of the extracted phenolic components, High-Performance Liquid Chromatography (HPLC) was employed. HPLC is widely recognized as one of the most effective methods for analyzing plant substances such as phenols due to its sensitivity and efficiency. The analytical HPLC system used consisted of a reversed phase HPLC with a silica-based C18 column (Agilent Technologies, Santa Clara, CA, USA), including a SPD-10A UV-VIS detector, VP pump LC-10AT, auto injector SIL-10AF, and system controller SCL-10A VP. The Chiralcel® OD-RH analytical column (150 mm × 4.6 mm diameter, 5 mm particle size; Chiral Technologies Inc., Exton, PA, USA) was utilized. The mobile phase employed was a mixture of acetonitrile, water, and phosphoric acid (30:70:0.08, v/v/v) under isocratic conditions, at ambient temperature (25 ± 1°C), with a flow rate of 0.4 mL/min. Each run took approximately 8 minutes, followed by a 15-minute cleanup. The compounds separated were detected by the built-in SPD-10A UV-Vis detector at a wavelength of 288 nm. Skendi et al's method [28] was employed to identify the phenolic compounds. By comparing the retention times of the standards (Table 1) with those of the peaks detected in the extract, the identity of each compound was determined.

2.7. DPPH Radical Scavenging Activity Assay

Amiri et al. [29] conducted a study in which they employed DPPH (2,2-diphenyl-1-picryl hydrazyl), a widely recognized organic compound, to evaluate its efficacy in eliminating free radicals, with minor modifications. They employed the DPPH assay for free radicals and the spectrophotometric technique to measure the radical-scavenging activity. The fractions were prepared using varying concentrations of (12.5, 25, 50, 100, and 200) µg/mL, which were subsequently dissolved in 1 mL of ethanol. To this mixture, 20 mg of DPPH dissolved in 100 mL of ethanol was added. After thorough shaking, the solution was left undisturbed at room temperature in darkness for 30 minutes. The control test utilized a DPPH solution, while ascorbic acid, a water-soluble vitamin, served as the reference standard. The antioxidant activity was determined using a UV-Visible spectrophotometer at 517 nm. The researchers employed the following equation to calculate the extent of radical scavenging:

DPPH Inhibition % = [(Ao − A1)/Ao] × 100
where Ao represents the absorbance of the control test after 30 minutes, and A1 signifies the absorption of the sample extract after 30 minutes.

2.8. Determination of cytotoxicity

The research aimed to investigate the cytotoxic impact of phenolic compounds and fatty acids derived from *U.dioca* by employing specific parameters. The MTT assay, a cell functional assay utilized to determine cell viability, and the high content screening (HCS) technique for cell apoptosis were employed. Cells were subjected to varying concentrations of the extracted compounds to discern their mechanisms of action.

2.9. MTT Assay

This study employed a colorimetric assay using 3-[4, 5–dimethylthiazoyl]-2, 5-diphenyltetrazolium bromide (MTT dye) to evaluate the viability of two cell types: Hepatocellular human carcinoma (HepG-2 cell line) and Normal human hepatic cells (WRL-68). To conduct the assay, 100 µl of cell suspension was added to each well of a flat-bottomed microculture plate for both cell lines. Subsequently, the cells were treated with 100 µl of extract, allowed to incubate for 24 hours, and then centrifuged to remove dead cells. Each well received 100 µl of 2 mg/ml MTT dye, and the experiment was left to proceed for an additional 4 hours. Afterward, 50 µl of DMSO solubilization solution was added to each well. The experiment was repeated three times. Once the dye was completely dissolved, the absorbance of the resulting colored solution, derived from viable cells, was measured at 620 nm using an ELISA reader. The average absorbance for each group of replicates was calculated. The percentage of cells that remained alive after exposure to different treatments was determined using the formula:
%Cell Viability = [(Mean Absorbance of treated sample / Mean Absorbance of non-treated sample) × 100].

In all experiments, the control group consisted of cells cultured in the medium without any treatment. This study was conducted at the Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, Center for Natural Product Research and Drug Discovery [30].

2.10. The High Content Screening (HCS) Assay

The utilization of the HCS kit in this assay enabled the simultaneous measurement of five distinct indicators of cell health. These indicators encompassed cell loss, alterations in nuclear size and shape, DNA content, changes in cell membrane integrity and permeability, mitochondrial membrane potential, and the location of released cytochrome c. One specific cell line, the human liver cancer cell line (HepG-2), was subjected to treatment with phenolic compounds and fatty acids extracted from U.dioca leaves at varying concentrations of 12.5, 25, 50, 100, and 200 µg/ml for a 24-hour period. It is important to note that the passage number of the cells used was 5. The HCS kit consisted of several components, including Cytochrome C Primary Antibody, DyLight649 Conjugated Goat Anti-Mouse IgG, Mitochondrial Membrane Potential Dye, Permeability Dye, Hoechst Dye, Wash Buffer, Permeabilization Buffer, Blocking Buffer, and a thin sealed plate. For the preparation of HepG2 cells, the protocol was optimized based on the instructions provided by ATCC (American Type Culture Collection), Production No. HB-8065. The study was conducted at the Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, Center for Natural Product Research and Drug Discovery [31].

2.11. Statistical Analysis

The data were subjected to statistical analysis in order to determine significant differences in the concentrations of active compounds extracted from Nettle leaves and their impact on both HepG2 cancer cells and normal cells. Statistical analysis was performed using a one-way analysis of variance (ANOVA) test, followed by comparisons of the arithmetic means using the DNCN polynomial test. Significant differences were considered at a probability level of 0.05 ($P \leq 0.05$) and a more stringent level of 0.01 ($P \leq 0.01$).

3. Results and Discussion.

3.1. Composition of fatty acids in U. dioica

Based on the results obtained from the GC-MS analysis, it was determined that Urtica dioica L. leaves contain five saturated fatty acids. The composition of fatty acids in Urtica dioica leaves is presented in Table 2. The predominant fatty acid identified in the nettle leaf parts during the study was Palmitic acid (16:0) at a percentage of 1.96%. Following closely, Behenic acid (22:0) emerged as the second most significant fatty acid, accounting for 0.71% of the total. Additionally, Stearic acid (18:0) and Pentacosylic acid (15:0) were found in notable quantities, representing 0.38% and 0.11% respectively. Finally, a small proportion of Myristic acid (14:0) was detected, amounting to 0.08%. These findings are visually depicted in Figures 1 - 5. Fatty acids play a crucial role in maintaining a healthy body as they provide us with energy and serve as a source of fat-soluble vitamins, such as vitamins A, D, and E. While our bodies require these essential nutrients, we obtain them through the food we consume (32) (33). The leaves of Urtica dioica, commonly known as stinging nettle, contain a wide array of compounds including minerals, amino acids, phytosterols, lignans, carotenoids, phenolics, and fatty acids. Among the fatty acids present, both palmitic acid and stearic acid are saturated fatty acids that can be found in all parts of the plant, with palmitic acid being particularly abundant. Additionally, Urtica dioica contains small amounts of monounsaturated fatty acids, with notable ones being palmitoleic acid, oleic acid, gadoleic acid, and erucic acid. The plant also contains polyunsaturated fatty acids like linoleic acid and a-linolenic acid, as previous studies conducted by different researchers have confirmed (34) (35) (36).

Extensive research has been dedicated to Urtica dioica due to its numerous medicinal applications. This plant plays a vital role in the functioning of the immune system, the breakdown of cholesterol, the regulation of membrane structure, and the functionality of the brain. Furthermore, fatty acids have demonstrated their effectiveness in controlling low-density lipoprotein levels and combating various ailments such as skin diseases, arthritis, asthma, lupus erythematosus, heart diseases, cancer, inflammatory and autoimmune diseases, coronary heart disease, and high blood pressure [37,38]. The leaves of nettles underwent comprehensive chemical analysis to further investigate their properties. Throughout the analysis, it was found that unsaturated fatty acids were more abundant than saturated ones, with polyunsaturated fatty acids, including linolenic, palmitic, and cis-9,12-linoleic acetates, being the predominant unsaturated fatty acids present [39,40].

3.1. Composition of Phenolic Compounds in U. dioica Fractions

Two fractions were obtained from Urtica dioica. Fraction I was extracted using ethyl acetate, while fraction II was extracted using ethanol. The compounds present in each fraction were determined by comparing the retention times and peak areas of phenolic compounds with those of corresponding standards. In Fraction I, a major peak corresponding to p-coumaric acid was identified using the standard. Fraction II showed two prominent peaks, namely vanillic acid and quercetin, as revealed in Figure 6. Nettle leaves are known to contain a diverse range of natural phenolic compounds, including flavonoids, phenolic acids, anthocyanins, and other phenols [50 ,51]. Extensive phytochemical studies have confirmed the abundance of phenolic compounds in nettle leaves [52]. Among these compounds, flavonols and their glycosides constitute the largest group of phenolics found in nettle. Identification of other compounds was likely achieved through the analysis of their mass spectra and fragmentation patterns, which had been previously reported. Notably, compounds such as quercetin and coumaric acid are classified as aglycones. Both flavonoids and phenolic compounds are common classes of natural products frequently encountered in various herbal sources [53]. p-Coumaric acid is formed by the attachment of a phenyl group to a single hydroxyl group in cinnamic acid. Among the different types of coumaric acids, p-coumaric acid is the most prevalent. It is abundantly present in numerous fruits, vegetables, and grains. When cinnamic acid undergoes an oxygenation process, it is transformed into p-coumaric acid. This compound exists in three distinct isomeric forms: o-coumaric, m-coumaric, and p-coumaric (p-

CA) acids. Of these isomers, p-coumaric acid (p-CA) is the most commonly encountered in nature [54,55].

Extensive research has demonstrated that both Vanillic acid and its metabolite, vanillyl, exert distinct effects on the human body. Vanillic acid has been found to inhibit the activity of 5'-nucleotidase in a highly specific manner. Similarly, vanillin, vanillin acid, and vanillyl alcohol are known to possess antioxidative properties and exhibit anti-inflammatory effects. Vanillic acid is specifically employed in the treatment of neurological and brain-related disorders. Its therapeutic action includes the reduction of beta-amyloid production, a significant contributor to Alzheimer's disease under conditions of oxidative stress, as evidenced in relevant models [56,57]. Quercetin, a flavonoid present in various plants utilized for culinary and medicinal purposes, exhibits a wide range of pharmacological properties. This phytochemical has been associated with numerous beneficial effects, including antioxidative, anti-inflammatory, antimicrobial, and anti-allergic properties. Furthermore, it has demonstrated chemopreventive, anti-genotoxic, and anti-tumor activities [58,59].

3.2. Antioxidant Activities of U.diocia Extraction

The results presented in Figure 7 illustrate the varying abilities of different fractions of U.dioica to eliminate DPPH radicals. Among the components, Fraction II exhibited a more potent antioxidant effect than the others, surpassing the standard. Following closely behind were Fractions I. Conversely, fatty acids demonstrated a weaker impact compared to the standard. Each fraction in this study exhibited antioxidant properties; however, their strengths varied due to the distinct redox properties of phenolic compounds. These compounds act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Notably, Fraction II, which consisted significantly of Vanillic acid and quercetin, exhibited a remarkably similar antioxidant effect to the standard, as indicated in Table 4.

Extensive previous research indicates that the effectiveness of phenolic compounds as anti-radicals and antioxidants is influenced by various factors. A crucial element is the number of hydroxyl groups directly attached to the aromatic rings, which significantly affects the stability of the hydroxyl radical subsequent to the transfer of hydrogen atoms from phenolics[60]. Additionally, the choice of solvents employed for plant extraction has been shown to impact DPPH results, aligning with the findings of this study. Analyzing the DPPH results, it becomes evident that Nettle possesses notable antioxidant properties due to its abundance of active compounds, including carotenoids, vitamins, fatty acids, and phenolic compounds. Nettle extracts, available in water, methanol, and alcohol, exhibit robust antioxidant capabilities, thereby serving as viable replacements for food antioxidants. These extracts effectively counteract the damaging effects caused by free radicals [61,62].The antioxidant effectiveness of vanillic acid, coumaric acid, and quercetin was assessed, revealing their potent antioxidant properties. These compounds are renowned as some of nature's most powerful antioxidants, providing protection against free radicals through direct displacement of the radical itself. Their interaction with the most reactive portion of the free radical enables them to effectively neutralize its harmful effects [63,64]. Furthermore, the fatty acids present in the n-Hexan extract exhibit antioxidant activities due to their electron-rich nature. These electrons act as donors, counteracting free radicals by transferring electrons from the fatty acids to the radicals, thereby transforming them into inactive compounds [65].

Table 1. Standards of phenolic compounds and their retention time

Standards	Retention Time (min)	Concentration (ppm)	Area [1]
p-Coumaric acid	3.523	25	1161,583,252 (0.01)
vanillic acid	3.031	25	445,452,319 (0.01)
Quercetin	3.261	25	12,367,103 (0.01)

[1] Area represented as mean (n = 5) with coefficient of variation in brackets

Table 2. Fatty acids separated from nettle leaves using GC-MS

No.	Common name of fatty acid	Chemical nomen-clature	R.t (min)	The area under curve%	The area under curve
1	Myristic acid (tetradecanoic)	C 14:0	8.926	0.08	3127428
2	Pentacosylic (pentacosanoic)	C 15:0	9.580	0.11	4221321
3	Palmitic acid (hexadecanoic)	C 16:0	11.423	1.96	76351495
4	Stearic acid (octadecanoic)	C 18:0	13.632	0.38	14782841
5	Behenic acid (docosanoic)	C 22:0	25.980	0.71	27763072

Table 3. Phenolic compounds in tow fractions and their retention time.

Fractions	No. of Peak	R .t (min)	Conc. (ppm) [c]	Identifed Compounds
I[a]	1	3.513	17.0 ± 0.6	p-Coumaric acid
II [b]	1	3.021	2.5 ± 0.3	vanillic acid
	2	3.220	2.4 ± 0.2	Quercetin

[a] Fractions identified from ethyl acetate extraction; [b] Fractions identified from ethanol extraction, [c] Values represent mean and standard deviation (n = 3)

Table 4. Antioxidant and free radical scavenging activity of isolated fractions

Concentration (µg/mL)	Standard%	Fraction I (%)	Fraction II (%)	Fatty acids (%)
12.5	17.63 ± 4.155	24.46 ± 1.696	17.40 ± 2.429	17.63 ± 2.024
25	40.43 ± 4.088	40.55 ± 2.461	36.96 ± 2.855	18.98 ± 2.715
50	54.48 ± 1.393	52.20 ± 2.144	54.48 ± 1.556	32.52 ± 0.8364
100	69.48 ± 2.141	63.85 ± 2.049	63.43 ± 2.086	42.79 ±1.036
200	80.36 ± 1.656	74.07 ± 2.762	75.58 ± 3.373	49.77 ±6.158
IC50	21.04	70.74	28.89	18.73

Table 5. Cytotoxic effect of *U. dioica* extraction on HepG2 and WRL68 by an MTT test for 24 hours at 37°C

Con. (µg/mL)	Fraction I (%)		Fraction II (%)		Fatty acids (%)	
	HpG2	WRL68	HpG2	WRL68	HpG2	WRL68
12.5	95.68±2.65	94.83±1.18	95.25±1.73	95.06±1.65	95.18± 1.28	95.95± 1.03
25	9.51±2.11	95.02±0.93	94.06±1.08	94.64±1.63	95.72± 0.81	95.22±0.82
50	79.05±7.34	94.37±1.45	74.34±4.14	93.79±0.24	90.70± 3.18	95.33±1.18
100	57.48±4.64	91.86±1.00	53.90±5.88	92.98±1.35	71.49± 3.40	93.60±2.10
200	39.45±2.37	79.01±2.26	38.93±1.01	82.60± 1.74	48.03± 2.55	84.80±1.20
400	27.70±3.36	71.41±1.10	31.79±4.48	76.89±1.82	39.35±4.78	71.95±0.81

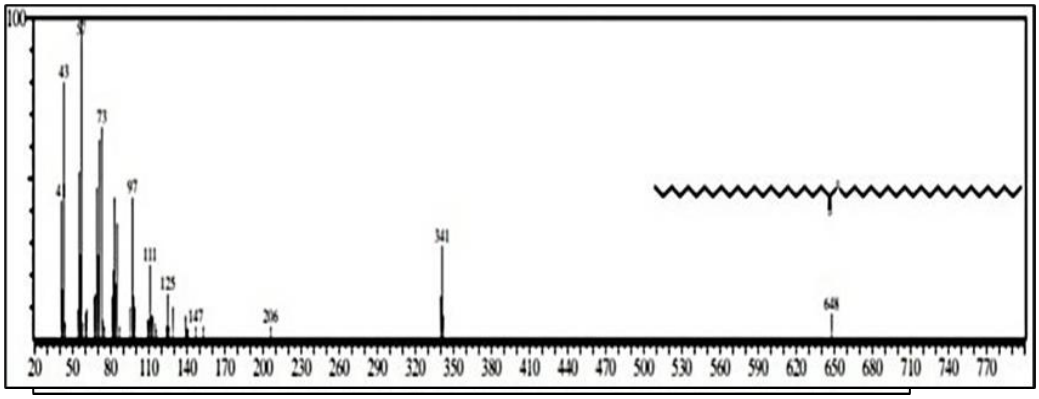


Figure 1. The curve and the structural formula of the compound Myristic acid

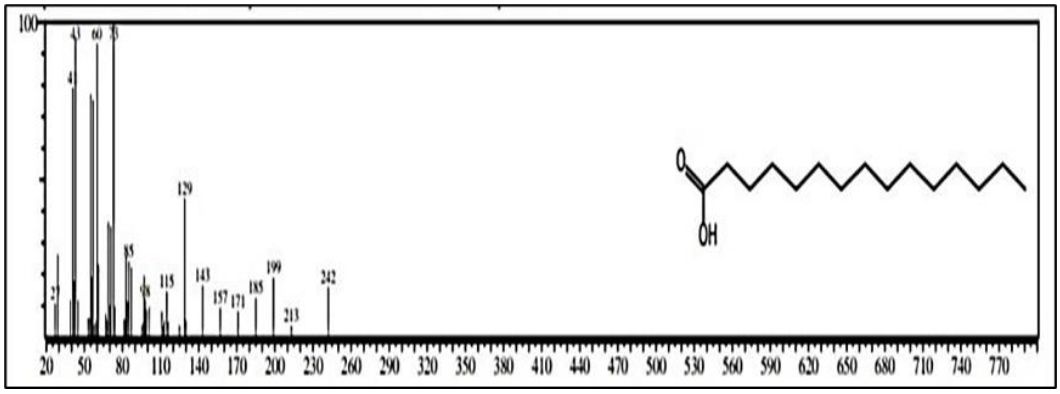


Figure 2. The curve and the structural formula of the compound Pentadecanoic acid

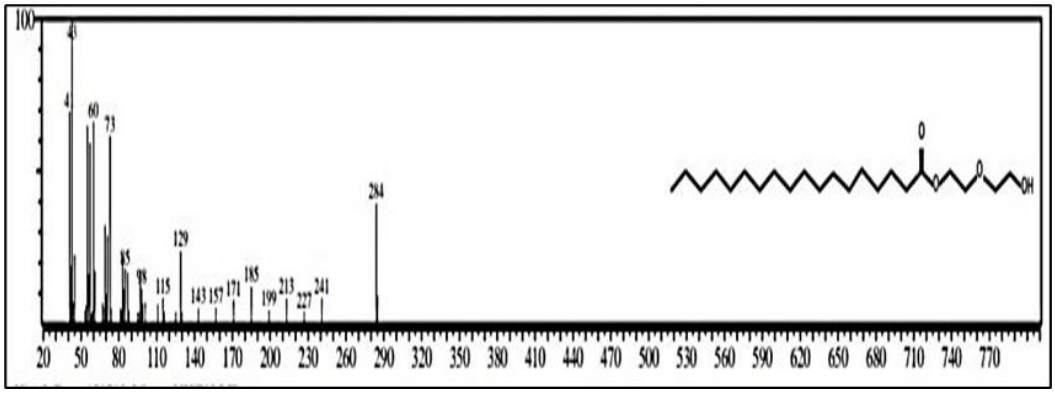


Figure 3. The curve and the structural formula of the compound Palmitic acid

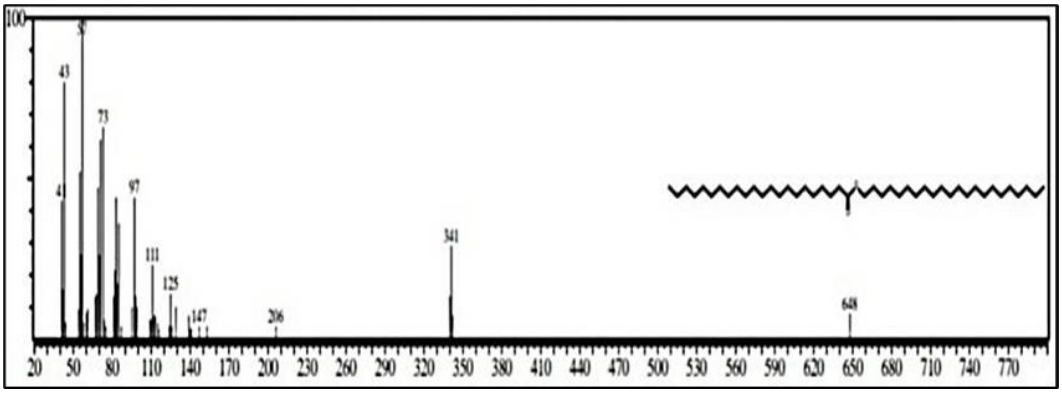
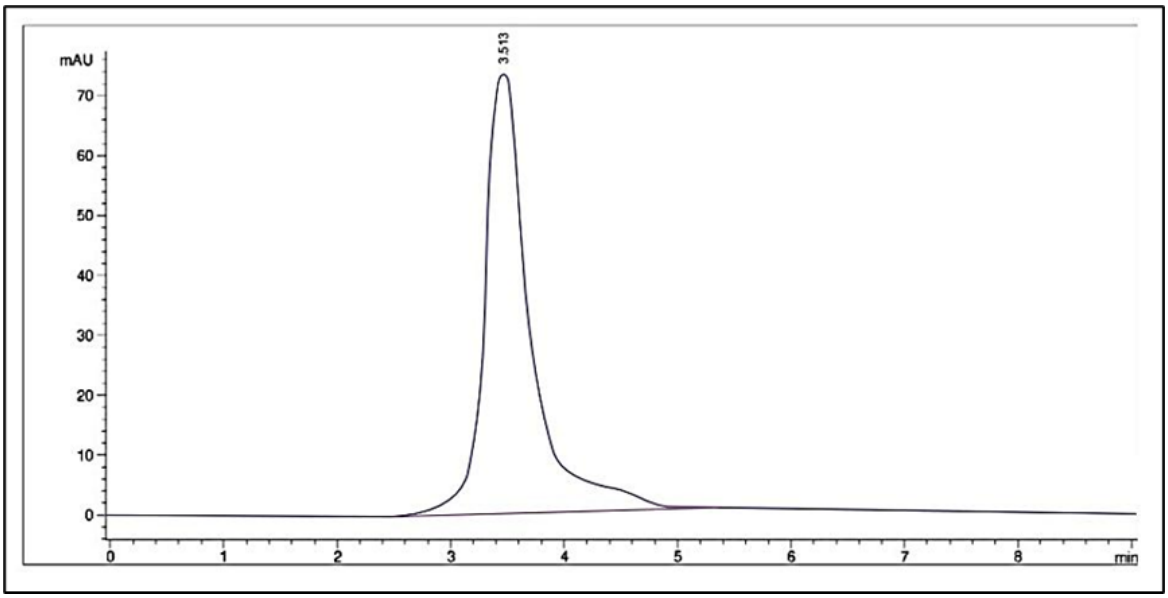
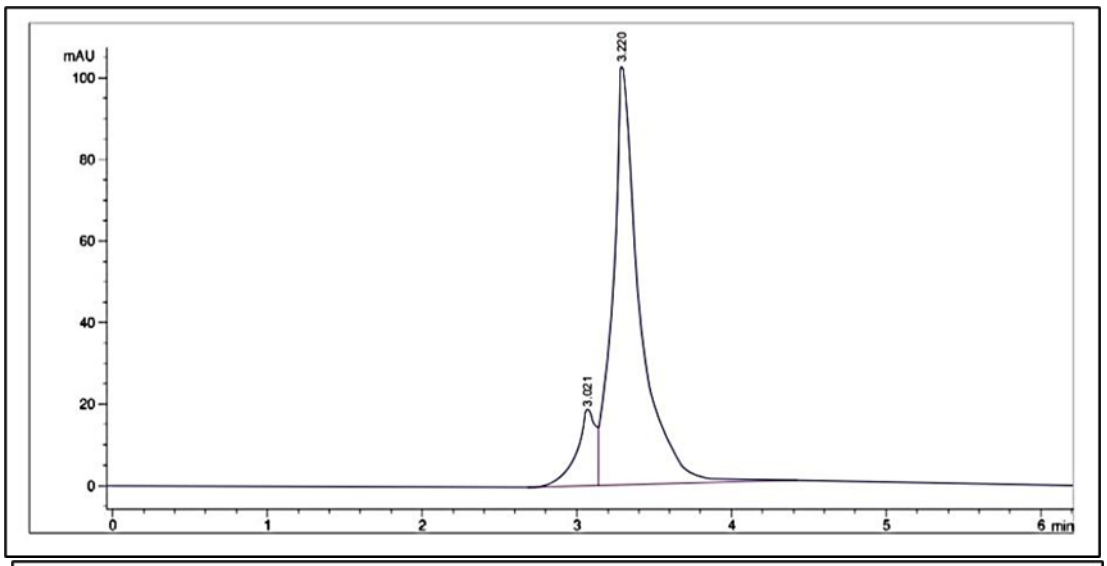


Figure 4. The curve and the structural formula of the compound Stearic acid



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.513	VB	0.3917	797.81285	76.35118	100.0000
Totals :				797.81285	76.35118	

Figure 5. The curve and the structural formula of the compound Behenic acid



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.021	VB	0.1258	872.37685	18.70055	40.5380
2	3.220	VV	0.2079	1279.62153	103.10608	59.4620
Totals :				2151.99838	121.80663	

Figure 6. HPLC chromatogram of Fraction I-II.

3.3. Cytotoxic Effect of U.dioica Extraction

Table 5 illustrates the outcomes of treating HepG2 and WRL-68 cells with fraction I, fraction II, and fatty acid extract derived from U. dioica leaf. When administered at a low concentration, the growth of cancer cells was halted, and as the concentrations of the tested compounds increased, inhibiting cancer cell growth became more attainable. Notably, at fraction I concentrations of 400 and 12.5 µg/ml respectively, the cell viability of cancer cells stood at 27.70±3.36% and 95.68±2.65%, while the corresponding viability for normal cells was 71.41±1.10% and 94.83±1.18%. Similarly, for fraction II, the cell viability of the HepG2 cell line was 31.79±4.48% and 95.25±1.73% at 400 and 12.5 µg/ml respectively, compared to WRL-68 with 76.89±1.82% and 95.06±1.65%. Finally, the fatty acid extract displayed a cell viability of 39.35±4.78% and 95.18±1.28% for the HepG2 cell line, while affecting WRL-68 with a viability of 71.95±0.81% and 95.95±1.03% at the same concentrations of 400 and 12.5 µg/ml respectively.

HepG2 cell lines have become a popular choice as experimental models due to their extensive usage and well-defined characteristics in liver cancer research. These cell lines serve as essential reference models in pharmaceutical studies, aiding in the development of novel drugs and providing valuable insights into drug metabolism processes [66]. It is noteworthy that over 70% of current anticancer drugs are derived from natural sources [67], with some of these compounds exhibiting remarkable properties as chemotherapeutic and chemopreventive agents. Not only are these substances generally well-tolerated, but they are also considered safe, easily accessible, and cost-effective [68]. U. dioica exhibits diverse biological activities, capable of modulating key cellular metabolic processes and regulating apoptotic pathways. Recent studies have highlighted its cytotoxic properties, particularly in combating various types of cancer, such as colon, stomach, lung, prostate, and breast cancers. U. dioica possesses compounds with potent antioxidant and free radical-scavenging properties, which contribute to reducing the elevated levels of oxidative stress observed in cancer cells and aiding in cancer prevention [69,70]. Conventional cancer treatments typically target the elimination or inhibition of cancer cell growth. However, it has been observed that individuals with cancer exhibit lower rates of apoptosis. Therefore, an effective cancer treatment should induce apoptosis in cancer cells, leading to the selective demise of cancerous and damaged cells while sparing normal dividing cells. Studies have demonstrated the significant apoptotic and cytotoxic effects of key compounds, such as p-coumaric acid, quercetin, vanillic acid, and other phenolic compounds found in plants.

4. Conclusions

The aim of this research is to extract phenolic compounds and fatty acids from Nettle leaves and explore their antioxidant and anticancer properties using various chromatography methods. The antioxidant activity of the phenolic compounds was assessed using the DPPH test, yielding significant antioxidant effects. Among the identified phenolic compounds, Part II exhibited the highest percentage of antioxidant activity at 75.58% concentration of 200 µg/ml, while Part I demonstrated an antioxidant effect of 74.07% at the same concentration. In comparison, the fatty acids displayed a comparatively lower antioxidant efficacy of 49.77% at the mentioned concentration. Notably, ascorbic acid showed an antioxidant effect of 80.36% at the same concentration, outperforming the phenolic compounds and fatty acids. In the MTT cytotoxicity assay conducted on the hepatocellular carcinoma cell line HepG2 and the normal cell line WRL68, Part I revealed that HepG2 exhibited the highest inhibition rate of 72.3% at a concentration of 400 µg/ml, while displaying no significant toxic effect on the WRL68 normal cells. In Part II, the phenolic compounds demonstrated a lower inhibition percentage of 68.21% on the cell line HepG2. On the other hand, the fatty acids exhibited lower efficacy, with a 60.65% inhibition of cell proliferation at the mentioned concentration.

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