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Chemical Composition of *Pulicaria undulata* (L.) Methanolic Extract and Its Potential Antioxidant and Cytotoxic

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Abstract

Natural products play a vital role in the development, design, and discovery of new medications, while also serving as a key source of drugs and biologically active compounds. This study investigates the chemical composition, antioxidant, and cytotoxic properties of the methanolic extract of *Pulicaria undulata* (L.). Utilizing GC-MS analysis, we identified various bioactive compounds, including fatty acids and terpenoids, contributing to its therapeutic potential. Antioxidant activity was evaluated using DPPH and ABTS assays, revealing concentration-dependent scavenging effects, with IC_{50} values of 38.46 mg/mL (DPPH) and 33.51 mg/mL (ABTS). Additionally, cytotoxic effects were assessed on human tumor cell lines (HePG-2, MCF-7, PC3), demonstrating selective cytotoxicity, particularly against HePG-2 cells (IC_{50} : 36.59 µg/mL) while sparing normal WI-38 fibroblasts (IC_{50} : 100 µg/mL). These results emphasize the potential of *Pulicaria undulata* as a valuable source of antioxidants and anticancer compounds, encouraging further investigation for therapeutic uses.

Keywords: *Pulicaria undulata;* GC-MS; bioactive component; DPPH, Anticancer.

1. Introduction

In many developing countries, medicinal plants are the primary source of healthcare for the majority of the population. The World Health Organization (WHO) reports that approximately 80% of the global population depends on traditional medicine, primarily derived from plants, to meet their primary healthcare requirements [1]. Medicinal herbs have played a crucial role in healthcare systems worldwide for centuries, and they continue to hold significant importance in various aspects of human health and well-being [2]. Aromatic medicinal herbs are rich in various chemical compounds responsible for their biological activities in treating numerous human diseases. These include a wide array of secondary metabolites such as alkaloids, flavones, flavonoids, sesquiterpene lactones, diterpenes, triterpenes, naphthoquinones, anthocyanins, coumarins, catechins, isocatechins, and others, which exhibit antioxidant, anti-inflammatory, antimicrobial, and other health-beneficial effects [3].

Natural products are essential not only for the design, synthesis, and discovery of new drugs but also as a fundamental source of medications and bioactive substances. These natural compounds have been crucial in the development of therapeutic agents for various diseases. Antioxidants safeguard cells from oxidative damage by either scavenging free radicals or repairing damaged molecules. Adequate intake of antioxidants is believed to protect the body from various diseases, including neurodegeneration, aging, viral infections, and cancer [4-6].

Cancer remains a leading cause of death worldwide, accounting for 10 million fatalities in 2020, which translates to one in every six deaths globally. The most common types include breast, lung, colorectal, and prostate cancer, while lung, colorectal, and liver cancers are the primary causes of cancer-related deaths, responsible for 3.5 million fatalities—about one-third of all cancer deaths in 2020 [7]. Early detection and effective treatment can cure many cancers. Among the conventional treatment options, chemotherapy is one of the most commonly employed and effective methods. It functions by targeting tumor cells and producing reactive oxygen species to destroy these cells through genotoxicity. However, chemotherapy also impacts normal cells, causing a variety of dose-dependent side effects such as fatigue, nausea, vomiting, hair loss, and, in severe cases, even death [8-10].

Species of the *Pulicaria* genus (family Asteraceae) are widely distributed across Asia, Africa, and Europe and are renowned for their medicinal properties due to traditional applications worldwide. These plants are rich in bioactive metabolites, including mono-, sesqui-, and diterpenoids, as well as phenolic compounds and flavonoids [11, 12].

The Egyptian desert plant *Pulicaria undulata* (L.) (syn. *Pulicaria crispa* (Forssk.) Benth et Hook) is particularly notable for its traditional medicinal uses, such as treating diabetes, abscesses, cardiac and skin disorders, and chills [13]. In Egypt, it is also used as an herbal tea for inflammation and as an insect repellent [11]. Extracts and compounds from *P. undulata* have demonstrated various pharmacological activities, including antioxidant, neuroprotective, antiacetylcholinesterase, anticancer, and α-glucosidase inhibitory effects [14-17]. These activities are linked to its diverse chemical constituents, such as terpenes, flavonoids, essential oils, and sterols [18-20].

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Medicinal plants have been used as remedies in Egypt for thousands of years, with the ancient Egyptians demonstrating extensive knowledge of various medicinal herbs and their efficacy in treating a wide range of ailments. Even today, many of these plants remain integral to traditional medicine practices [21]. This study utilizes standard techniques, such as GC-MS spectroscopy and the DPPH assay, to analyze the antioxidant properties of *Pulicaria undulata*, along with the MTT assay to evaluate its cytotoxic effects.

2. Materials and Methods

2.1. Plant material and extraction process

During the flowering season in April 2023, several populations of *P. undulata* were collected from Egypt's northeastern desert. The identification was based on the works of Tackholm [22] and Boulos [23]. After being washed, the samples were cleaned and air-dried. 20 grams of the dried plant material were mixed with 200 ml of methanol in a 500 ml conical flask. The mixture was then shaken continuously in a water bath shaker at room temperature for two hours (Memmert WB19, Schwabach, Germany). The resulting mash was filtered using Whatman filter paper (No. 1, 126 mm, Cat. No. 1067 134, Germany). The concentrations of the final plant extracts were measured and stored at ±3 °C [24].

2.2. GC-MS analysis

The *P. undulata* plant extract was evaluated using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a TG-5MS direct capillary column (30 m x 0.25 mm x 0.25 µm film thickness) [25]. The column oven was initially set at 50°C and then increased at a rate of 5 °C per minute to reach 250 °C, where it was maintained for 2 minutes before being raised at a rate of 30 °C per minute to 300 °C for another 2 minutes. The temperatures for the MS transfer line and injector were set at 260°C and 270 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL/min. After 4 minutes, the solvent was removed, and an Autosampler AS1300 injected 1 µL of diluted samples into the GC in split mode. Mass spectrometry data were collected using electron impact (EI) at 70 eV, with a scan range of 50–500 m/z and an ion source temperature of 200 °C. The GC-MS analysis identified five components for each peak in the mass spectrum data of the extracted plant material, compared against WILEY 09 and NIST 14 databases. The suggested structures of the components were determined based on probability factors and primary fragmentation patterns.

2.3. Radical Scavenging Assay

The antioxidant efficacy of the MeOH extract from the aerial parts of *P. undulata* was evaluated by measuring its ability to scavenge DPPH (2,2-Diphenyl-1-picrylhydrazyl) radicals [26]. Solutions of extract were prepared at concentrations of 50, 100, 200, 300, 400, and 500 mg/L by diluting in methanol. A reaction mixture was created by rapidly mixing equal volumes of freshly prepared 0.3 mM DPPH solution with each concentration of extract and allowing it to react for 30 minutes at 25 °C. The absorbance of the resulting mixture was then measured using a spectrophotometer (Analytik Jena, Jena, Germany) at a wavelength of 517 nm. Ascorbic acid was used as a control, following the same procedure at concentrations of 1.0, 2.5, 5, 10, 15, and 20 mg/L. The DPPH radical scavenging activity (%) was calculated using the following equation:

$$\text{DPPH radical scavenging (\%)} = [(A_{control} - A_{sample})/\text{A control}] \times 100$$

Where $A_{control}$ and A_{sample} are the control and MeOH sample absorbances, respectively.

In accordance with Re et al. [27], the extract of P. undulata demonstrated antioxidant activity by reducing ABTS (2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic Acid) radicals. Similar to the DPPH assay, extract concentrations ranging from 50 to 500 mg/mL were prepared. Specifically, 2 mL of freshly prepared ABTS solution was mixed with approximately 0.2 mL of the EO sample, and the mixture was incubated in the dark for 6 minutes. The color absorbance of the resulting solution was measured using a Spectronic 22D spectrophotometer (Milton Roy, CA, USA) at a wavelength of 735 nm. A standard solution containing ascorbic acid was also tested. The percentage of ABTS radical inhibition was calculated using a formula similar to that used for the DPPH assay

2.4. MTT assay

The cytotoxic activity of the MeOH extract from *P. undulata* was assessed using an MTT assay (Applichem, USA). The study utilized distinct human tumor cell lines: HePG-2, PC3, and HeLa, which were obtained from ATCC, a Cairo-based company specializing in the production of biological products and vaccines. Doxorubicin, a commonly used chemotherapeutic agent for cancer treatment, served as a reference. This assay quantifies the reduction of MTT by mitochondrial dehydrogenase, resulting in the formation of a blue formazan product that indicates mitochondrial functionality and overall cell health.

Cultured cells, which doubled every 24 hours at a concentration of 2×10^4 cells/mL, were evenly distributed into 96-well plates from Greiner, Germany, with each well containing 200 µL of growth media. After incubating the plates for 24 hours, the plant extracts diluted in a solution containing 10% DMSO were added to the cell cultures, resulting in a final concentration of 4 mg/mL for the extracts. The cells were then cultured for 72 hours at 37°C in an incubator with 5% carbon dioxide, with various doses of the extracts administered to assess their effects.

Following the incubation period, 20 µL of phosphate-buffered saline (PBS) containing 5 mg/mL of MTT was added to each well. After the media was removed, the blue formazan crystals formed within the cells were dissolved in 100 µL of DMSO. The reduced MTT was quantified by measuring the absorbance at 540 nm using a microplate reader (Thermo Scientific Multiscan Spectrum). The cytotoxic effects of the EO were determined by comparing the optical densities of treated and untreated cells. The formula used to evaluate cytotoxicity relative to controls is as follows:

$$\% \text{ Inhibition} = \frac{\text{O. D. cont.} - \text{OD samp.}}{\text{OD cont.}} \times 100$$

Where the concentration (in μg/mL) that results in 50% cell death is referred to as IC_{50}.

2.5. Statistical Analysis

Experiments measuring antioxidant and cytotoxic potency were repeated three times with three replicates using the Costat software (CoHort Software, Monterey, CA, USA). One-way analysis of variance (ANOVA) was then performed on the results to see whether there were statistically significant differences between samples.

3. Results and Discussion

3.1. GC-MS Spectroscopy

The chemical characterization of the components extracted from *Pulicaria undulata* reveals a complex mixture of hydrocarbons, fatty acids, esters, and steroids, contributing to the plant's biological activity, including its antioxidant antimicrobial and anticancer potential. Table 1 identifies 22 distinct volatile components, covering 98.07% of the total area, based on gas chromatography analysis. Key components include hydrocarbons like 36-dimethyloctan-2-one, accounting for 1.93% of the total, and higher molecular weight compounds such as heptatriacontan-1-ol (3.29%). Notable fatty acids and esters include methyl oleate (24.57%), a major contributor to the extract's composition, and palmitic acid (9.68%), which is known for its role in skin health and other biological functions. Steroidal compounds, such as stigmast-5-en-3-ol, account for a smaller fraction (1.61%) but are significant due to their potential role in anti-inflammatory and antioxidant activities.

The high concentration of fatty acids, especially methyl oleate and palmitic acid, along with various hydrocarbons, may explain *P. undulata's* antioxidant properties. The diverse array of volatile components suggests that the extract's bioactivity is likely due to synergistic effects between these compounds, offering insights into its therapeutic potential for oxidative stress-related conditions. Several studies, including those by Abdallah et al. [20] and Al-Hajj et al. [28], have explored the characterization of volatile components in the extracts of *Pulicaria undulata*, highlighting the identification of long-chain fatty acids. Specifically, the volatile components of the petroleum ether extracted from the whole plant were analyzed using GC-MS spectroscopy, as reported by Elshiekh and Mona [29]. Additionally, Mansour [30] identified volatile compounds in P. undulata, with methyl linoleate (18.84%) being a notable fatty acid derivative.

Table 1. Chemical characterization of the components extracted from *P. undulata*.

No.	RT (min)	Volatile components	M.Wt.	M.Fw.	Area (%)
Hydrocarbons					
1	5.05	3,6-dimethyloctan-2-one	156.27	$C_{10}H_{20}O$	1.93
2	6.84	(2E,6E)-3,7-dimethylnona-2,6-dienal	166.26	$C_{11}H_{18}O$	2.17
3	10.91	tetradeca-1,13-dien-3-one	208.35	$C_{14}H_{24}O$	2.49
4	10.98	(Z)-2-butylocta-2,7-dien-1-ol	182.31	$C_{12}H_{22}O$	1.68
5	14.98	(1S,5R,9R)-10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5-ol	220.36	$C_{15}H_{24}O$	6.53
6	18.67	6,10,14-trimethylpentadecan-2-one	268.49	$C_{18}H_{36}O$	11.37
7	19.65	Retinal	284	$C_{20}H_{28}O$	1.63
8	32.87	3-ethyl-5-(2-ethylbutyl)octadecane	366.72	$C_{28}H_{58}$	1.49
9	35.13	Heptatriacontan-1-ol	537.01	$C_{37}H_{76}O$	3.29
Fatty acids and esters					
10	11.44	(Z)-7-methyltetradec-1-en-1-yl acetate	268.44	$C_{17}H_{32}O_2$	2.29
11	11.86	Ethyl (9Z,12Z)-octadeca-9,12-dienoate	308.51	$C_{20}H_{36}O_2$	2.51
12	20.02	Methyl 14-methylpentadecanoate	270.46	$C_{17}H_{34}O_2$	3.35
13	15.69	Ethyl (9Z,12Z)-octadeca-9,12-dienoate	308.51	$C_{20}H_{36}O_2$	2.52
14	15.8	Methyl (5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoate	318.5	$C_{21}H_{34}O_2$	3.93
15	21.1	Palmitic acid	256.43	$C_{16}H_{32}O_2$	9.68
16	22.65	Methyl (7E,10E)-octadeca-7,10-dienoate	294.48	$C_{19}H_{34}O_2$	1.97
17	22.75	Methyl oleate	296.5	$C_{19}H_{36}O_2$	24.57
18	23.73	Oleic acid	282.47	$C_{18}H_{34}O_2$	5.65
19	23.95	Myristic acid	228.37	$C_{14}H_{28}O_2$	3.28
20	29.41	Methyl docosanoate	354.6	$C_{23}H_{46}O_2$	2.27
21	34.65	(2-phenyl-1,3-dioxolan-4-yl)methyl (E)-octadec-9-enoate	444.66	$C_{28}H_{44}O_4$	1.86
Steroids					
22	33.08	Stigmast-5-en-3-ol	414.72	$C_{29}H_{50}O$	1.61
Total					98.07

RT: retention time, M.Wt.: molecular weight, M.Fw.: molecular formula.

2.2. Antioxidant Assay

Figure 1 compares the antioxidant activities of *Pulicaria undulata* methanol extract (*P. undulata* MeOH) and ascorbic acid, with their scavenging abilities measured against ABTS and DPPH free radicals at various concentrations. DPPH and ABTS assays are widely used methods to evaluate the antioxidant capacity of compounds, particularly in natural products and food

chemistry. Both assays are based on the ability of antioxidants to neutralize free radicals, which are unstable molecules that can cause oxidative damage in biological systems, leading to various diseases [31, 32]. The results show that the antioxidant activity of *P. undulata* increases with concentration, starting at 7.85% and 5.66% for ABTS and DPPH, respectively, at 50 mg/ml, and reaching 70.36% (ABTS) and 61.43% (DPPH) at 500 mg/ml. The IC_{50} values, which indicate the concentration required to inhibit 50% of free radicals, are 33.51 mg/ml for ABTS and 38.46 mg/ml for DPPH, showing that higher concentrations are needed to achieve significant antioxidant effects compared to the standard antioxidant, ascorbic acid.

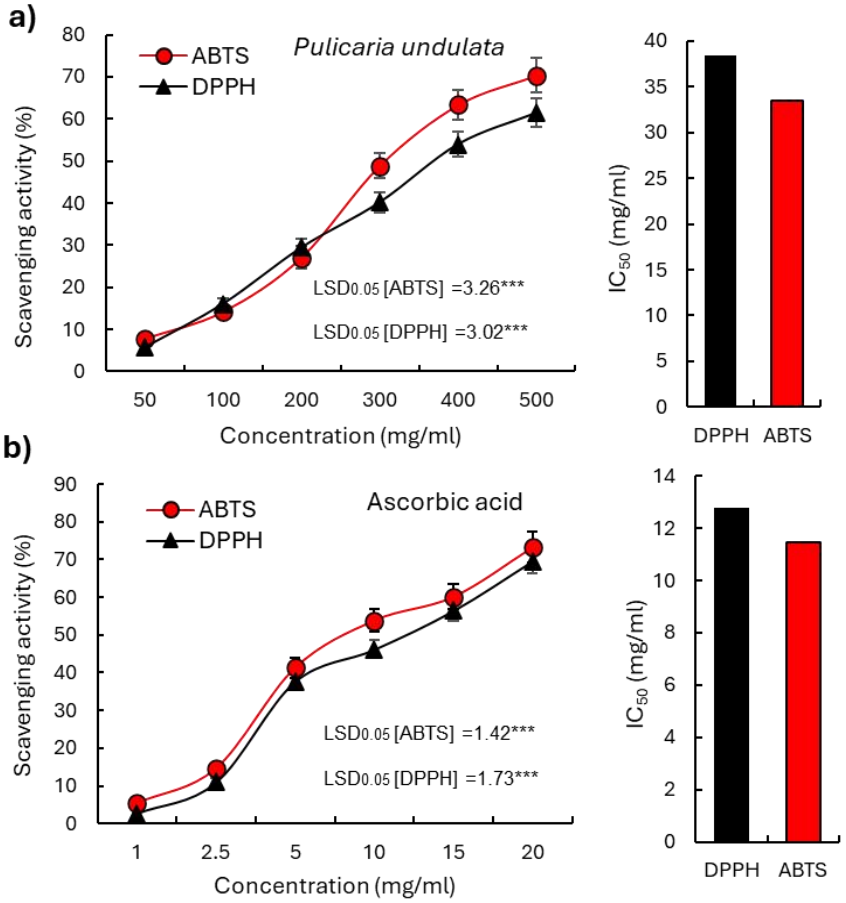

Figure 1. Antioxidant action of the *P. unduliata* MeOH extract (**a**) and ascorbic acid as a normal antioxidant (**b**). Quantities are means of triplicate ± stand. dev. IC_{50}: the total of sample needed to decrease the DPPH or ABTS absorbance by 50%. $LSD_{0.05}$ determined least significant (calculated by Factorial ANOVA).

Ascorbic acid demonstrates stronger antioxidant activity at much lower concentrations. For instance, at just 5 mg/ml, it shows 41.34% scavenging activity for ABTS and 37.68% for DPPH, much higher than *P. undulata* at the same concentration. The IC_{50} values for ascorbic acid are notably lower, at 11.47 mg/ml ABTS and 12.76 mg/ml for DPPH, indicating its superior efficacy in neutralizing free radicals. The differences in antioxidant efficiency between the extract and the pure compound may be due to the complex nature of the plant extract, which contains various phytochemicals acting together. The results obtained for *P. undulata* were in agreement with the findings of Mustafa et al. [17], who show that the oil extracted from *P. undulata*, growing wild in Egypt, contained major compounds, namely. All of these components demonstrated powerful antioxidant properties when tested by DPPH and ABTS assays.

The superior antioxidant activity was linked to the intrinsic antioxidant properties of the phytoconstituents, particularly their high concentrations. This is especially true for phenolic compounds, which are produced in abundance as a response to the harsh environmental variations in the plant's natural habitat [33-35]. The significant antioxidant activity of *P. undulata* is likely due to its phytochemical composition, which may include flavonoids, phenolics, and other compounds known for their free radical scavenging abilities [19,36,37]. The scientific literature has identified several phytochemical compounds in this plant that exhibit biological activity. These compounds include carvacrol, xanthoxylin and carvotanacetone [17], linalool, p-cymene and carvacrol [38,39].

2.3. Cytotoxic assay

The cytotoxic activity of *Pulicaria undulata* was evaluated against various tumor cell lines, including HePG-2 (liver cancer), MCF-7 (breast cancer), and PC3 (prostate cancer), as well as normal WI-38 fibroblast cells. The results demonstrated that P. undulata exhibited dose-dependent cytotoxicity across all tumor cell lines, with IC_{50} values of 36.59 µg/mL for HePG-2, 55.27 µg/mL for MCF-7, and 44.38 µg/mL for PC3 (Figure 2). These IC_{50} values indicate moderate potency of the plant extract against cancer cells, with the strongest cytotoxic effect observed against HePG-2 cells. In comparison, the IC_{50} value for normal WI-38 cells was 100 µg/mL, which suggests that *P. undulata* has selective cytotoxicity towards cancer cells while sparing normal cells at lower concentrations. For comparison, the chemotherapeutic agent doxorubicin was used as a standard. Doxorubicin demonstrated significantly higher cytotoxicity with much lower IC_{50} values across all tumor cell lines-7.38 µg/mL for HePG-2, 6.36 µg/mL for Hela, and 10.95 µg/mL for PC3 (Figure 2). These values highlight the potent antitumor effects of doxorubicin, as it is a well-established drug used in various cancer treatments. However, the IC_{50} for WI-38 normal cells was also 100 µg/mL, similar to that of *P. undulata*, indicating that while doxorubicin is highly effective, its therapeutic window may also affect normal cells at higher concentrations.

The comparison between *P. undulata* and doxorubicin underscores the potential benefits of the plant extract as an alternative or complementary anticancer agent. While *P. undulata* is less potent than doxorubicin, its selective toxicity towards cancer cells over normal cells makes it a promising candidate for further investigation. The fact that it causes less damage to healthy cells is particularly valuable, as one of the major challenges in cancer therapy is balancing efficacy against tumors with minimizing harmful side effects to normal tissues. The phytochemicals present in the extract, such as terpenes, flavonoids, and phenolic compounds, may contribute to its antioxidant and anticancer properties. Identifying and isolating these active compounds could enhance the extract's therapeutic potential and reduce the required dosage, thereby improving its efficacy while maintaining low toxicity to normal cells.

The cytotoxic mechanism of a substance is influenced by various factors, including the concentration of the sample, the nature of the extracted components, and the phytochemical composition. These factors play a significant role in determining the effectiveness of the extract in targeting cancer cells. Additionally, the specific type of tumor or normal cell line being targeted is another critical factor, as different cells respond uniquely to treatments. Cytotoxicity is often associated with the loss of cell protein, which can indicate cell damage or death, contributing to the overall mechanism through which cancer cells are affected [40-42].

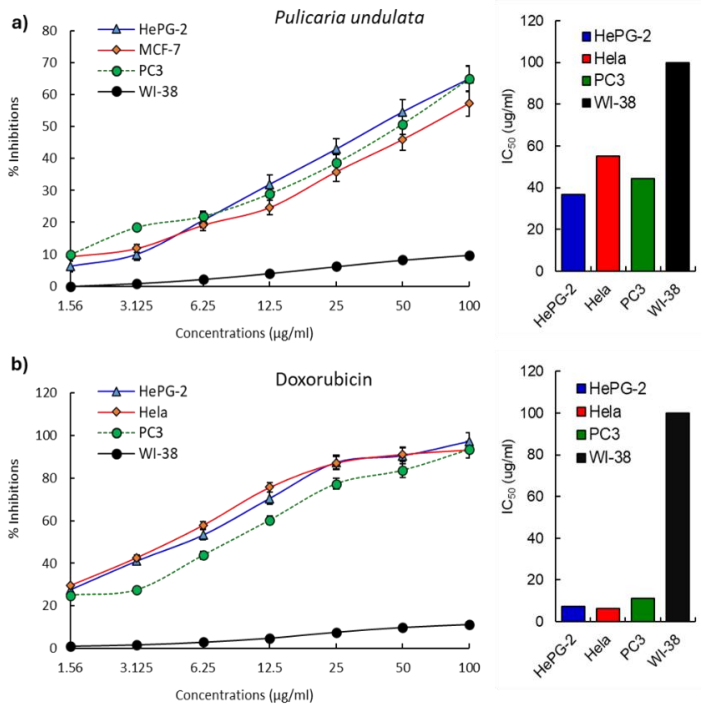

Figure 2: Cytotoxic action and the IC_{50} values of the prepared plant sample versus the cancer and ordinary cells (WI-38) at diverse applications, and doxorubicin as regular.

4. Conclusions

In conclusion, the study of *Pulicaria undulata* methanolic extract has demonstrated significant potential as a foundation of bioactive mixtures with notable antioxidant and cytotoxic properties. The chemical analysis via GC-MS identified a rich composition of fatty acids and terpenoids, which are likely responsible for the observed biological activities. The antioxidant assays indicated a concentration-dependent scavenging effect on free radicals, underscoring the extract's ability to mitigate oxidative stress.

Moreover, the cytotoxicity assessments revealed selective activity against human tumor cell lines, particularly highlighting its effectiveness against HePG-2 cells while sparing normal fibroblast cells. This selective cytotoxicity presents Pulicaria undulata as a promising candidate for further development in cancer therapeutics, offering potential advantages over conventional chemotherapeutic agents by minimizing harm to healthy tissues. These findings warrant additional research to isolate and characterize the active compounds within *Pulicaria undulata* and to explore their mechanisms of action.

Conflicts of interest

"There are no conflicts to declare".

Acknowledgments

"None"

5. References

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