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EXPLORATION OF THE ANTIOXIDANT AND ANTICANCER PROPERTIES OF METHANOLIC EXTRACTS DERIVED FROM FIVE DIFFERENT *JASMINUM* **SPECIES, ALONGSIDE AN ANALYSIS OF THE PHYTOCONSTITUENT PROFILE USING GC-MS**

Nelly M. George* , Nermeen Foda, Marwa A. Yassin and Marwa Eldemerdash

Department of Botany and Microbiology, Faculty of Science, Zagazig University, 44519, Zagazig, Egypt

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ABSTRACT. Several bioactive constituents found in conventional medicine possess antioxidant and anti-cancer effects and may be useful in the treatment of a variety of diseases, so the current study sought to conduct a thorough investigation of their potential use as a potent antioxidant and anticancer agent for five *Jasminum* species. The preliminary phytochemical analysis in a methanol extract of *Jasminum* indicated the existence of numerous bioactive secondary metabolites. More accurately, we discovered many novel compounds such deoxyspergualin, gentamicin B, cefazolin, cytidine, cycloheptanone oxime, folinic acid, 1-dodecene, epoxynonane derivative, dlcitrulline, thiocyanic acid, limonene 5,3',4'-trihydroxyflavone and 4-fluoro histidine. The results demonstrated that the plants have therapeutic values, since the extracts' antioxidant impact was examined using radical 2,2-diphenyl-1-picrylhydrazyl. The methanolic extract displayed varying half-maximal inhibitory concentration values slightly above the standard, indicating the significance of the bioactive metabolites present in the plant. Additionally, investigation was conducted on the potential anticancer properties targeting a specific liver cancer cell line and we revealed that *J. azoricum* was the powerful plant would effectively inhibit cancer cell lines at both 10 and 100 µg/mL.

KEY WORDS: *Jasminum*, Phytoconstituents, Anticancer, Antioxidant activity, GC-MS

INTRODUCTION

The botanical family Oleaceae comprises 28 genera and approximately 900 species, among which the genus *Jasminum* L. stands out with an estimated 200 species [1]. The distribution of Jasmine species spans various tropical regions worldwide, with a notable prevalence in Southeast Asia. The richness of Jasmine species is most prominent in regions of southwestern and Southeast Asia, although a limited number of wild species can also be found in Asia, Africa, Australia, the Pacific islands, and Europe. These species are believed to have undergone initial domestication in the ancient old-world period and can be observed in the new world in their wild form [2]. The Oleaceae family is utilized to prepare numerous ayurvedic formulations, mainly for wound care (Jati Gitra) and mucosa wounds in the mouth (decoction is employed for gargle) [3, 4].

Plants generally exhibit antioxidant characteristics, with a robust and varied assortment of metabolites, including several classes of flavonoids and non-flavonoids [5, 6]. Variations in structure, hydroxyl (OH) groups, and the specific positioning of metabolites play a pivotal role in influencing the divergence observed in antioxidant activity and biological effects [7]. Both herbs and vegetables, particularly wild species, contain antioxidants and a variety of phytochemicals that can delay oxidative stress and other related issues [6].

The percentage of active constituents in secondary compounds determines their potential for use in medication formulation. Gas chromatography mass spectroscopy (GC-MS) is an effective system for separating and identifying complex mixtures of metabolites [10]. In recent years, gas

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^{*}Corresponding authors. E-mail: nellymichel@zu.edu.eg

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chromatography-mass spectroscopy (GC–MS) was directed to influence the structures of different phytoconstituents [11]. The generation and dispersion of reactive oxygen species (ROS) alters the composition and function of cell membranes. When the mitochondrial respiratory chain fails, free radicals begin to damage polyunsaturated fatty acids in biological membranes [12, 13]. In such situations, an antioxidant-containing molecule may be advantageous in limiting ROS generation and lowering oxidative cell damage, particularly if the antioxidant action is mediated by a functional extract [14]. Biological molecules, such as lipids, proteins, and DNA, are predominantly susceptible to degradation due to the impact of reactive oxygen species (ROS), ultimately leading to cellular mortality [15]. Plant constituents can be employed to balance out the extent of antioxidants as well as free radicals in the body and may be a less risky alternative to synthetic antioxidants [16]. Because of the multiple negative effects of artificial antioxidants, the world is actively looking for a potentially safe medicinal plant to treat a variety of oxidative stress-related illnesses [17]. Plants possess basic antioxidant properties that facilitate associating a few categories of flavonoids (flavones, flavanols, and anthocyanins) and non-flavonoids (lignin's, phenolic acids, stilbenes, and terpenoids), contributing to the compounds biological and antioxidative activity [18, 19].

According to data from the World Health Organization (WHO), fatalities from cancer are predicted to increase by 104% worldwide by 2020, with poorer countries anticipated to have the largest increase (70%). Chemotherapy is a therapeutic method that is frequently used to treat cancer. With approximately 27 primary forms and an alarming death rate of 8.2 million people yearly, cancer is one of the deadliest diseases [20]. It is well known that chromosomal aberrations, oncogene activation, and oxidative DNA damage caused by free radicals play a role in the development and spread of cancer [21].

This research endeavor seeks to establish a correlation between the conventional therapeutic applications of jasmine, the components present in extracts derived from individual plants, and their antioxidative as well as anticancer properties. More specifically, the focus is placed on the characterization of phytoconstituents through the utilization of the gas chromatography-mass spectrometry (GC-MS) technique and the evaluation of the biological effects of the identified compounds sourced from the foliage extract of the plant. As a result, a variety of compounds exhibiting antioxidative and anticancer characteristics were isolated from five distinct botanical species, along with the revelation of their respective chemical structures.

EXPERIMENTAL

Chemicals, reagents and equipment

Standard methods were used for drying the solvent and all procedures were achieved in an inert environment. 85% methanol modified Eagle Medium, 100 mg/mL of streptomycin, 100 units/mL of penicillin, and 10% bovine serum were obtained from the Department of Botany Microbiology Zagazig University. *Jasminum* plant was collected and purchased from Orman garden in Egypt. Sonicator and spectrophotometer were obtained from the Department of Botany Microbiology, Zagazig University, A capillary column TG–5MS (30 m x 0.25 mm x 0.25 μ m film thickness) was utilized for chemical composition analysis of the samples using the Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA).

Plant material and extraction procedures

A whole wild population of *Jasminum* plants was acquired (Figure 1). After gathering the plants, they were properly cleaned with tap and then distilled water. After blotting them with blotting paper, they were pounded into a fine powder and allowed to dry at 37 °C in the dark. After homogenizing roughly 100 g of powder, it was macerated in 1000 mL of 85% methanol in a

stoppered container. Additionally, for a traditional extraction, it was allowed to stand at room temperature for a day. The extract and powder were then placed in a sonicator at 4 °C for 30 min. After that, rotary vapor was used to filter and concentrate this extract under vacuum at 40 $^{\circ}$ C, yielding crude extract.

Table 1. List of cultivated *Jasminum* species used in the present study and their accession code, location sites and habitat.

No.	<i>Jasminum</i> species	Accession code	Collection sites	Habitat
	J. grandiflorum			
	J. azoricum		Botanical garden	Semi shaded area
	<i>J. sambac</i> (doble flower),	JSD	in Orman, Egypt	and sunny area
	<i>J. sambac</i> (single flower)	JSS		
	J. nudiflorum			

Identification of alkaloid constituents by gas chromatography–mass spectrometry (GC-MS) analysis

A capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness) was utilized for chemical composition analysis of the samples using the Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA). Initially, the column oven temperature was set at 50 °C, then gradually raised by 5 °C/min to 250 °C with a 2-min hold, followed by a rapid increase of 30 °C/min to reach 300 °C, where it was maintained for 2 min. The injector and MS transfer line temperatures were held at 270 and 260 °C, respectively, while a constant helium flow rate of 1 mL/min served as the carrier gas. Samples diluted to 1 µL were injected automatically through an Autosampler AS1300 connected to a GC in split mode, featuring a 4-min solvent delay. EI mass spectra were acquired at 70 eV ionization voltages within the m/z range of 50–650 using full scan mode. The ion source temperature was fixed at 200 $^{\circ}$ C, and the identification of components was based on comparison of retention times and mass spectra with WILEY 09 and NIST 14 mass spectral database entries [22].

Determination of antioxidant activity

DPPH assay

The radical scavenging activity of DPPH radical 2,2-diphenyl-1-picrylhydrazyl and the antioxidant activity of *Jasminum* plants were evaluated in triplicate utilizing the Boly *et al.* [23], Soxhlet extraction was utilized in the procurement of powdered leaves utilizing methanol as the solvent. The measurement of absorbance (Abs) was executed at a specific wavelength of 540 nm. After this, data analysis was performed utilizing Microsoft Excel®, and the radical scavenging activity was quantified as a percentage of inhibition by applying the continuity formula:

where A_0 = the absorbance of control. A_1 = the absorbance of standard.

% Inhibition = $A_0 - A_1/A_0 \times 100$ (1)

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Determination of anticancer activity

Liver cancer cell line (HEPG2)

Nawah Scientifc Inc. provided cancer cell lines (Mokatam, Cairo, Egypt). At 37 °C, the cells were incubated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin, and 10% heat-inactivated fetal bovine serum within a

humidified environment containing 5% (v/v) CO₂. Following this, the Sulforhodamine B (SRB) assay was employed to assess cellular viability [23, 24].

RESULTS AND DISCUSSION

The manifestation of diverse phytoconstituents has been informed with different structures, and types present in different *Jasminum* species, GC-MS analysis for *J. grandiflorum* indicate that there are different phytoconstituent the peaks were identified as distinct entities, each exhibiting varying levels of relative abundance. (deoxyspergualin, gentamicin B, cefazolin, cytidine, cycloheptanone oxime and folinic acid) illustrated in Figure 1-A, Table 2, for *J. azoricum* (1 dodecene) illustrated in Figure 1-B, Table 3, for *J. sambac* (JSD) (cefazolin, dl-citrulline, thiocyanic acid and limonene) illustrated in Figure 1-C, Table 4, for *J. sambac* (JSS) (5,3',4' trihydroxyflavone and 4-fluoro histidine) illustrated in figure 1-D Table 5 and for *J. nudiflorum* (cytidine and cefazolin) illustrated in Figure 1-E and Table 6. All the important phytocosnstituent characterize each species shown in Figure 2.

When looking to the number of phytoconstituent we found that it varied among five species where it was 25 for *J. grandiflorum* and 18 for *J. azoricum* and 12 for *J. sambac* (JSD) and 4 for *J. sambac* (JSS) and 16 for *J. nudiflorum* where the variation in number of phytoconstituents could be used as tool to differentiate between plant species.

Table 2. List of phytoconstituents extracted from *J. grandiflorum* identified by GC-MS analysis illustrating phytoconstituents, retention time, molecular weight and molecular formula.

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Figure 1. Relative abundance of methanol extracts of the selected leaves of (A) for *J. grandiflorum.* (B) For *J. azoricum.* (C) For *J. sambac* (Doble flower). (D) For *J. sambac* (single flower). (E) For *J. nudiflorum* (single flower).

N _o	Rt.	Phytoconstituents	Molecular	Molecular
			formula	weight
1	3.29	Pyrrolidinyl dene amino	$C_6H_{10}N_2O_2$	142
\overline{c}	3.92	Bromopride	$C_{14}H_{22}BrN_3O_2$	343
3	4.49	Hexaborane	B_6H_{10}	76
4	5.74	Epoxynonane	$C_{13}H_{26}N_2O_4$	142
5	7.51	-6Deoxy-D-mannono-4-lactone	$C_6H_{10}O_5$	162
6	7.69	Dodecyl acrylate	$C_{15}H_{28}O_2$	240
7	9.23	-1Nonanol	$C_9H_{20}O$	144
8	9.35	1H-Benzotriazole, 1-methyl	$C7H7N3$	133
9	9.72	-2Trifluoroacetoxydodecane	$C_{14}H_{25}F_{3}O_{2}$	282
10	10.88	Gentamicin B	$C_{19}H_{38}N_{4}O_{10}$	482
11	10.98	Benzeneethanamine, N,à,à-trimethyl	$C_{11}H_{17}N$	163
12	11.73, 13.68,	-1Dodecene	$C_{12}H_{24}$	168
	14.60, 15.65, 17.34			
13	17.49	Aziridine, 2-methyl-2-(2,2,4,4-	$C_5H_9DO_2$	103
		tetramethylpentyl)-		
14	19.05	-1Octanol, 2,7-dimethyl	$C_{10}H_{22}O$	158
15	19.50	-2Methylmalonic acid	$C_4H_6O_4$	118
16	20.68	-3MethylundecanE-6,6-D2	$C_{12}H_{24}D_2$	172
17	22.27	-1,2 Epoxy nonane	$C_9H_{18}O$	142
18	31.93	Allylcyclopentane	C_8H_{14}	110

Table 3. List of phytoconstituents extracted from *J. azoricum* identified by GC-MS analysis illustrating list of phytoconstituents extracted from *J. azoricum* identified by GC-MS analysis illustrating phytoconstituents, retention time, molecular weight and molecular formula.

Table 4. List of phytoconstituents extracted from *J. sambac* (JSD) identified by GC-MS analysis illustrating phytoconstituents, retention time, molecular weight and molecular formula.

Table 5. List of phytoconstituents extracted from *J. sambac* (JSS) identified by GC-MS analysis illustrating phytoconstituents, retention time, molecular weight and molecular formula.

Figure 2. List of some important phytoconstituent resulted from five different *Jasminum* species imported from GC-MS analysis each one labelled with their accession code illustrated in Table 1.

N ₀	Rt.	Phytoconstituents	Molecular formula	Molecular weight
	3.51	Methanone, 1,3-dithian-2-ylphenyl	$C_{11}H_{12}OS_2$	224
\mathfrak{D}	3.92	Benzene, 1-chloro-2-methyl	C ₇ H ₇ Cl	126
3	4.17	Cytidine	$C_9H_13N_3O_5$	243
4	4.28, 7.65, 12.29, 14.07	Benzeneethanamine, N,à,à-trimethyl	$C_{11}H_{17}N$	163
$\overline{5}$	4.49	-3Pentanol, 2,4-dimethyl	C ₇ H ₁₆ O	116
6	5.86	1H-Benzotriazole	$C_6H_5N_3$	119
7	7.50	á-Alanine	$C_3H_7NO_2$	89
8	9.68	-2Phenylbutanamide	$C_{10}H_{13}NO$	163
9	10.85	1(2H)-Naphthalenone, 3,4-dihydro	$C_{10}H_{10}O$	146
10	15.13, 15.18	-1,2Diethylcyclobutane	$C_8H_{10}D_6$	118
11	18.01, 22.45	Hexaborane (10)	B ₆ H ₁₀	76
12	18.45	-2Methylmalonic acid	$C_4H_6O_4$	118
13	18.66	-3Methylundecane-6,6-D2	$C_{12}H_{24}D_2$	172
14	20.02	N,N-Dimethyl-1,2,3-trithian-5-amine	C ₅ H11NS ₃	181
15	21.21	Cefazolin	$C_{14}H14N_8O_4S_3$	454
16	22.27	-2Undecenoic acid	$C_{11}H_{20}O_2$	184

Table 6. List of phytoconstituents extracted from *J. nudiflorum* identified by GC-MS analysis illustrating phytoconstituents, retention time, molecular weight and molecular formula.

Antioxidant assay

Free radical scavenging activity in five Jasminum extract by DPPH assay

In *J. grandiflorum* the DPPH assay demonstrated that the percentage of inhibition rises proportionally with escalating concentrations. Specifically, at a concentration of 100 µg/mL, the inhibition reached 54.58%, whereas at 800 µg/mL, it increased to 90.25%. A correlation analysis between the concentration levels and inhibition percentages was conducted to ascertain the IC_{50} value, which was determined to be 52.16 (Table 7) Figure 3.

In *J. azoricum* the DPPH assay demonstrated that the percentage of inhibition rises in tandem with escalating concentration levels. Specifically, at a concentration of 100 µg/mL, the inhibition reached 44.78%, whereas at a concentration of 800 µg/mL, the inhibition surged to 91.33%. A correlation between concentration and inhibition percentage was illustrated graphically to ascertain the IC_{50} value which was determined to be 33.86 (Table7) Figure 3.

In *J. sambac* (JDS), the DPPH assay demonstrated that the level of inhibition rises proportionally with escalating concentrations. Specifically, at a concentration of 100 μ g/mL, the inhibition reached 36.25%, whereas at 800 μ g/mL, it surged to 85.65%. A correlation analysis between concentration levels and inhibition percentages was conducted to ascertain the IC_{50} value resulting in an IC_{50} value of 216.95 (Table7) Figure 3.

In *J. sambac* (JSS), the DPPH method unveiled a positive correlation between the percentage of inhibition and the rising concentration levels. Specifically, at a concentration of $100 \mu g/mL$, there was a 48.55% inhibition, whereas at 800 μ g/mL, the inhibition reached 89.99%. A graphical representation of the concentration versus inhibition percentage was constructed to ascertain the IC₅₀ value, which was determined to be 55.42 (Table 7) Figure 3.

In *J. nudiflorum*, the DPPH assay demonstrated that the percentage of inhibition rises proportionally with the ascending concentration levels. Specifically, at a concentration of 100 µg/mL, the inhibition reached 38.55%, whereas at 800 µg/mL, it reached 77.36%. A correlation between the concentration levels and inhibition percentages was established through graphical representation to ascertain the IC₅₀ value, which was calculated to be 223.86 (Table 7) Figure 3.

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Anticancer activity for HEPG2: Liver cancer

The rise in percentage of inhibition is positively correlated with higher concentrations. The association between concentration levels and percentage of inhibition is demonstrated in the study. Table 7 viability of HEPG2: Liver cancer cell line was 48.65 ± 0.99 at conc. 10 μ g/mL but 6.50±0.12 at conc. 100 µg/mL *J. grandiflorum*, was 41.85±0.89 at conc. 10 µg/mL but 2.25±0.10 at conc. 100 µg/mL for *J. azoricum*, was 89.25±0.68 at conc. 10 µg/mL but 25.32±0.12 at conc. 100 µg/mL for *J. sambac* (JDS), was 54.25±0.95 at conc. 10 µg/mL but 4.32±0.25 at conc. 100 µg/mL for *J. sambac* (JSS) and was 88.35±0.78 at conc. 10 µg/mL but 23.25±0.25 at conc. 100 µg/mL for *J. nudiflorum*.

Correlation between antioxidant and anticancer activity

According to the results, out of the five different extracts of jasmine, three (JA, JG, and JSS) have high antioxidant and anticancer properties, respectively, in comparison to Trolox, which has an IC₅₀ of 16.13. The other two extracts, however, have low antioxidant potential and anticancer properties, and we also discovered that *J. azoricum* is the better plant with medicinal therapeutic compounds.

The separation of all metabolites in the samples examined was achieved through GC-MS analysis, resulting in the generation of a representative spectral output. Each metabolite was expected to exhibit a distinct spectral peak, with retention time aiding in the differentiation between them. The magnitude of the peaks corresponded to the concentration of the associated compounds in the specimens under analysis. GC-MS has revealed the presence of numerous secondary metabolites in various plant species, such as phenols, alkaloids, flavonoids, glycosides, terpenoids, and tannins [26].

There are several and different phytoconstituent analyzed by gas chromatography (GC) and GC/mass spectrometry (MS) in different parts of the worlds reported by [27-31]. In *J. grandiflorum* we found several phytoconstituent such as folinic acid which reported and considered as potential agent for cancer therapy [32, 33]. In *J. azoricum* we found dodecene as antimicrobial, antifungal and anticancer platforms [34]. In *J. sambac* (D) dl-citrulline and limonene regarded as anticancer agents, [35]. Trihydroxyflavone have both anticancer and antioxidant activity [36]. Finally in *J. nudiflorum* cefazolin which considered as antibiotic having antimicrobial and anticancer activities [37].

According to Suaputra et al., (2021) [38] and George et al. (2021, 2022 and 2023) [6, 22 and 39], plants containing phytochemicals possess potential as antioxidants and anticancer agents. Utilizing Jasmine plant extract and the comparison standard of torolx, researchers determined

antioxidant capacity through the calculation of IC_{50} . In a study conducted by Rivero-Cruz et al. (2021) $[40]$ IC₅₀ represents the concentration necessary for a substance to inhibit 50% of biological activity. This metric was employed in the research to assess the antioxidant activity present in the jasmine plant extract. A lower IC_{50} value indicates a higher level of antioxidant capacity within the extracts derived from jasmine plants.

Numerous human health issues, such as inflammation, carcinogenesis, neurological diseases, and aging, are primarily caused by oxidative stress. Presumptive experimental proof links the generation of ROS to life damage, even if numerous factors contribute to the genesis of these diseases. This information may potentially give a routine basis for therapy [41-44].

Many natural products' effects on health as well as the in vivo protective role of natural antioxidants obtained from medicinal plants or added to food appear to have significantly changed recently. These antioxidants guard against oxidative damage brought on by reactive oxygen species (ROS) [45]. In the present study, the antioxidant activity of extracts of plants were

assessed using three different methods (DPPH scavenging activity) of methanolic extracts at different concentrations (200-1000 µg/mL).

Trolox reagent was used as a standard and its activity was evaluated under the same conditions as the extracts revealed that the IC_{50} of standard was slightly lower than the sample indicate its efficacy in scavenging free radicals, have powerful antioxidant activity, with fascinating insights for their future valorization as pharmaceuticals. The DPPH technique is based on the measurement of antioxidant scavenging potential towards the stable DPPH radical. This method offers a quick and easy to evaluate the anti-radical activities of antioxidants, as the radical compound is stable and would not need to be generated as in other radical scavenging techniques [46].

The scavenging activity of free radical is measured by the ability to quench the stable DPPH radical. The assay gave data on the reactivity of examined extract with a stable free radical. *Jasminum* extracts were able to quench the DPPH-radical and had nearly equivalent ability. The five extracts content have different level of activity where the most potent extract has antioxidant activity were *J. azoricum* which equal 33.86 this may have phytochemical constituent which are more active than other extracts and according to [47]. The ratio of active constituent determines the potential of its uses for drug preparation, and this is elucidated in all extracted metabolites, and this is great evidence that plant metabolites are very excellent and potential source for drug preparation and treatment of diseases. Generally, antioxidants (vitamins C, E, carotenoids, and polyphenols) are important for good bone health. They neutralize reactive particles called free radicals that are associated with all inflammatory and painful phenomena [48, 49]

In the present study, different plant extracts revealed cell proliferation inhibition and cytotoxicity in liver cancer cell line (HEPG2) in a dose- and time-dependent way without impacting normal cells reflecting anti-proliferative action for *J. azoricum* at 10 µg/mL was 41.85±0.89 then *J. grandiflorum* at 10 µg/mL was 48.65±0.99. Natural phytochemical decrease genotoxicity, improve anti-inflammatory and antioxidant activity, suppress proteases and cell proliferation, and safeguard intracellular communications to influence signal transduction and apoptosis pathways [50].

Cell shrinkage, resulting from membrane blebbing and DNA fragmentation, are some of the alterations observed. Among these effects, the initiation of apoptosis stands out as the primary mechanism through which malignant cells are eradicated. Apoptosis represents a form of programmed cell death characterized by distinctive morphological features and chromatin condensation. It is plausible to posit that the inhibitory effect of the extract on the proliferation of liver cancer cells (HEPG2) stems from its ability to trigger apoptosis. Prior investigations into the aqueous extract of Jasminum have highlighted its capacity to impede cellular proliferation and colony formation across various cancer types such as colorectal, prostate, cervical, and breast cancers. This extract not only suppresses cellular growth but also enhances the process of apoptosis [50].

CONCLUSION

This study concludes by highlighting the medicinal potential of bioactive chemicals found in five species of *Jasminum*, with an emphasis on their anticancer and antioxidant qualities. Numerous unique chemicals were discovered in the methanol extracts by phytochemical analysis, indicating a rich profile of secondary metabolites with potential therapeutic uses. The antioxidant potential of these extracts was validated by the DPPH assay; *J. azoricum* exhibited the strongest activity, as evidenced by its low IC50 value. Additionally, against liver cancer cell lines, *J. azoricum* showed notable anticancer effectiveness, particularly at doses of 10 and 100 μ g/mL, highlighting its potential as a natural source for the development of antioxidant and anticancer medicines.

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