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Original Article

In vitro Evaluation of Antioxidant, Antibacterial, Antifungal and Cytotoxic Activities of *Johreniopsis stricticaulis* in Two Growth Stages

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ABSTRACT

Johreniopsis stricticaulis (Rech.f.) Pimenov is a rare aromatic species of the Johreniopsis genus (Apiaceae family). In this research, the aerial part of the plant was harvested in two phonological stages (pre-flowering [PF] and after-flowering [AF]), and different solvent extracts (MeOH, ethyl acetate and CH_2Cl_2) were prepared. Phenolic compounds, antioxidant activity, antibacterial and antifungal properties were investigated. Cell cytotoxicity was also evaluated on three cell lines as human prostate cancer cell line (PC3), human breast cancer cell line (MCF-7) and mouse fibroblast cell line (L929; as a non-malignant cell line). The results showed that the phenolic contents in different extracts varied significantly. Methanol extracts had the highest phenolic content and also antioxidant activity in both stages, but it was lower than other extracts on cell death. Antimicrobial evaluation of the extracts showed that ethyl acetate extract was found to be the most effective, followed by dichloromethane and methanol extracts. All extracts were more effective in Gram-positive bacteria (*Staphylococcus aureus*) than Gram-negative bacteria (*Escherichia coli*) and fungi (yeast) (*Candida albicans*) and higher biological activity of *J. stricticaulis* extracts were achieved when the plant was harvested at after flowering stage. For getting higher biological activity of *J. stricticaulis* extract harvesting the plant after the flowering stage was recommended.

INTRODUCTION

Medicinal and aromatic plants produce a diverse array of bioactive compounds known as active substances which are known as secondary metabolites. These compounds create health benefits beyond basic nutrition [1]. Clinical microbiologists are also interested in the antimicrobial plant extracts. Antimicrobial activity of medicinal plants depends on harvest time (phonological stage), method of extraction and solvent type (solvent polarity) [2]. In recent years, there has been a growing scientific interest in identifying effective and non-toxic compounds that exhibit antioxidant properties. Numerous studies have assessed the antioxidant activities of both herbal and synthetic substances. The secondary metabolites derived from plants are known to confer various health benefits to humans and animals, as well as to inhibit food spoilage. This research aims to discover new natural sources of antioxidants and antibacterial agents from indigenous plant species.

Medicinal plants have been considered as a promising source for finding new anticancer compounds. Discovery of new molecules from plants which show new innovative mode of action in cancer cells have been considered as a topic of interest for cancer researchers. Medicinal plants of Apiaceae family produce a wide range of bioactive compounds that show medium to high antioxidant and antimicrobial activity for example Cumin (*Cuminum cyminum*) and Black Zira (*Bunium persicum*) products (oils as well as their aqueous and solvent-derived extracts) have shown significant antioxidant and antimicrobial activity in several test methods [3]. Genus of *Johreniopsis* which belongs to tribe of Peucedaneae, subfamily of Apioideae and

family of Umbelliferae (Apiaceae) has four species (*J. oligactis*, *J. scoparia*, *J. seseloides*, and *J. stricticaulis*) in Iran [4,5]. *J. stricticaulis* is one of the Iranian species of *Johreniopsis* and to the best of our knowledge, there have not been any studies carried out on *J. stricticaulis*, so in the present work biological properties of various extracts of the species grown wild in Iran were studied.

MATERIALS AND METHODS

Plant Material and Extracts

The aerial parts of *J. stricticaulis* were collected during the pre-flowering (PF) and after-flowering (AF) stages from Khorasan province of Iran in May 2018. A voucher sample of the plant (43265 FUMH) was deposited at the Ferdowsi University of Mashhad Herbarium. For the preparation of extracts, dried aerial parts of the plant were ground and successively extracted with various solvents such as ethyl acetate (ETOAC), dichloromethane (CH_2Cl_2) and methanol (Me-OH). The extraction procedure was conducted using the maceration technique at room temperature and in the absence of light. Subsequently, a concentration gradient varying from 0.5 to 2.5 mg/ml was established.

Evaluation of Antioxidant Activity and Total Phenolic Content

The antioxidant activity of the extracts was evaluated by two methods: 2,2- diphenyl-1- picryl-hydrazyl (DPPH) free radical and Ferric reducing antioxidant power (FRAP assay). Initially, various concentrations of the extracts were formulated, ranging from 0.5 to 2.5 mg/ml. In DPPH method, 100 µL of each extract (2-75 mg/ml) was reacted with DPPH reagent (0.16 mM, 100 µL) in a 96-well microliter plate for 30 min in dark condition, the absorbance was read at 490 nm by microplate reader ((BioTek, USA) [6]. The Butylated hydroxytoluene (BHT) was used as a reference compound. The radical scavenging was calculated by:

$$Inhibition\ (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

In FRAP assay, 100 µL from each extract was added to FRAP reagent. The mixture was vortexed and incubated at 30 °C for 4 minutes. The solution's absorbance was read at 593 nm with microplate reader. Aqueous solution of $FeSO_4.7H_2O$ (0-1 mM) was used for calibration curve. Antioxidant activity was expressed as mmol Fe (II)/g extract [7].

For determination of total phenolic content,100 mL of Folin-Ciocalteu's phenol reagent (0.2 N) was added to ten-microliter of each extract in 96-well plate. After 3 min, 90 mL of saturated sodium carbonate was added to the mixture and incubated at room temperature for 1h. The absorbance of samples was measured at 630 nm using a ELISA reader (BioTek, USA). The standard curve was built using Gallic acid (31.25 to 500 mg/ml). The total phenolic content was expressed as micrograms of Gallic acid equivalents per g of dried extract [8].

Antimicrobial Assay

The three micro-organisms used in this study were *Escherichia coli* ATCC (8739), *Staphylococcus aureus* ATCC (6538) and *Candida albicans* ATCC (10231). These micro-organism strains were procured from Pasteur Institute of Iran. Bacterial strains were grown in Mueller-Hinton broth at 37 °C for 24h and fungal strain was grown in soybean casein agar at 25 °C for 24h [9]. The antimicrobial activity was assayed by disc and well diffusion, minimal inhibitory concentration (MIC) and minimal bactericidal- fungicidal concentration (MBC-MFC) using the micro-dilution broth techniques. In disc diffusion method, discs containing extracts with 6 mm diameter were placed on the plates with 100 mm diameters containing Mueller Hinton agar (MHA) for bacterial strains and soybean casein agar (SCA) for fungal strain with the 1.5×10^8 micro-organism cells. The extracts were dissolved in DMSO to give 100 mg/ml. Fifty µL of each sample was placed on the discs and then incubated at 25-37 °C for 24-48h. In well diffusion method, wells with 6 mm diameter into the agar plates were spread with bacteria at a 10^8 $CFU.ml^{-1}$ (Colony Forming Unit) density. 50 µL from each extract (100 mg/ml) was added into the wells and allowed to diffuse at room temperature for 2h. The plates were incubated at 37 °C for 24h and then zone of inhibition was measured (mm) [9]. Gentamycin and Nystatin were used as positive controls and DMSO was used as negative control [10]. The MIC values of the plant extracts were determined by the micro-dilution assay in 96 multi-well micro-titer plates according to the National Committee for Clinical Laboratory Standards. Each sample was diluted in DMSO and Mueller Hinton Broth and Soybean Casein Broth to give a concentration of 100-0.195 mg/ml. Then, 200 µL of each concentration was added in a well into 96-well

micro-plate containing 20µL from 1.5×10^6 CFU/mL of micro-organism. The micro-plate was incubated at 25-37 °C for 24-48h. After incubation, 20µl of 2,3,5 tri-phenyl-tetrazolium chloride (TTC) was added to each well as a colorimetric indicator of bacterial and fungal growth and incubated for 60 minutes at 37 °C. The MIC was determined as the lowest concentration of the sample that showed no color change [11]. To determine MBC/MFC, 10µLwas taken from each well put in Mueller Hinton Agar and Soybean Casein Agar for 24h at 25-37 ºC respectively. The highest dilution that yielded no signal colony on the solid medium was taken as MBC/MFC [11, 12].

Cytotoxic Effects

Three cell lines; human prostate cancer cell line (PC3), human breast cancer cell line (MCF-7) and mouse fibroblast cell line (L929; as a non-malignant cell line) were obtained from Pasteur Institute (Tehran, Iran). The cells were maintained at 37˚C in a humidified atmosphere 95% containing 5% CO_2. Cytotoxic activity was performed by using MTT assay (3- (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide) as previously described [13]. Various concentrations of extracts (12.5-200 µg/mL) with cells were plated in a 96-well culture plate. The cultured plates were incubated for 24, 48, and 72h at 37˚C and 5% CO_2; then 20 µL of MTT solution in phosphate-buffered saline (PBS) was added to each well at a final concentration of 0.5 mg/ml followed by further incubation for 3h at 37˚C. Cell morphology also was studied before adding MTT by light microscope. The medium was removed; 100 mL of DMSO was added to each well for solubilizing the formazan. The absorbance was measured at 490nm using ELISA reader (BioTek, USA). Doxorubicin was used as a positive control [14,15]. All analyses were done in triplicate. The inhibition was calculated using the following formula:

$$Inhibition\ (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

A control: Absorption of control or blank sample; A sample: Absorption of test samples

Statistical Analysis

Each disc diffusion and minimum inhibitory concentration (MIC) assay was carried out with a minimum of three repetitions for each sample. In the disc diffusion analysis, three to six measurements were taken for every bacterium to ensure the reliability of the results, and the mean ± standard deviation was presented. IC_{50} was determined by Graph Pad Prism software (Version 5) [6,16]. The analysis of the variations among the tested extracts was assessed through one-way analysis of variance (ANOVA), which was subsequently complemented by the Tukey test to conduct additional multiple comparisons, with a significance level set at a probability of $P\leq 0.05$.

RESULTS AND DISCUSSION

Extract yield

The comparison of extract yields is presented in Table 1. The data indicates that the ethanolic extract obtained during the pre-flowering stage yielded 10.43%, whereas the extract from the post-flowering stage exhibited the lowest yield at 1.9%. The analysis reveals that there is no notable distinction between the samples taken during the pre-flowering and post-flowering stages when methanol is employed as the solvent, indicating that methanol does not significantly influence the results in this context.

Phenolic Contents

The phenolic contents in different extracts varied significantly ($p\leq 0.05$). The results of the total phenolic content of *J. stricticaulis* in two growth stages range from 1297.60 to 31466.0 µg of GAE/g extract (Table 1).

Table 1 Yield, total phenolic, FRAP and DPPH radical scavenging activity for the different extracts of *J. stricticaulis*.

Extract	Yield (w/w %)	Total phenolic (µg of GAE/g extract)	FRAP (mmol/g extract)	IC_{50} (DPPH) (mg/ml)
MeOH (PF[a] stage)	3.5 b	31466.0 ± 0.0035 *	537.41 ± 0.037	0.58 ± 0.003
ETOAC (PF stage)	10.43 a	2919.6 ± 0.0007	-**	4.48 ± 0.01
CH_2Cl_2 (PF stage)	2.4 bc	6812.4 ± 0.0007	-	2.46 ± 0.004
MeOH (AF[b] stage)	4.06 b	14598.0 ± 0.015	0.43 ± 0.0	0.86 ± 0.003
ETOAC (AF stage)	1.9 c	10056.44 ± 0.03	0.2 ± 0.008	1.09 ± 0.008
CH_2Cl_2 (AF stage)	2.2 bc	1297.60 ± 0.02	0.216 ± 0.004	8.3 ± 0.008
BHT[c]	-	-	-	0.3 ± 0.002

* Data are mean ± SD values, **No activity, [a]Pre-flowering, [b]After-flowering, [c] Butylated hydroxytoluene

Methanol extract had the highest phenolic content in two stages. Also, the results showed that MeOH extracts at PF stage had higher total phenol content. Variations in yields and phenolic contents of various extracts are due to the polarities of different compounds present in the plant [17].

Antioxidant Activity

The findings indicated that the methanol extract exhibited superior radical scavenging activity at two specific stages, although this was not consistent across all concentrations (Fig 1). The data demonstrated IC50 values ranging from 0.58 ± 0.003 mg/ml to 8.3 ± 0.008 mg/ml, which were notably lower than those observed for synthetic antioxidants. The antioxidant properties of medicinal plants are particularly important due to the detrimental effects of free radicals on human health and food quality [18]. Pammi *et al.,* (2023) demonstrated that different extracts of medicinal plants showed different antioxidant activity [19]. Sabaragamuwa and Perera (2023) compared the different extracts of *Centella asiatica*. Their exploration of various solvent systems, particularly those incorporating water and methanol, provided valuable insights into the extraction dynamics of total triterpene content (TTC), total polyphenolic content (TPC), total flavonoid content (TFC), and the fluctuations in the antioxidant capacity of the resulting extracts. This research demonstrated that both ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) significantly enhance the efficiency and effectiveness of bioactive compound extraction, optimizing factors such as yield, extraction duration, and the conservation of solvents and energy. Additionally, their findings indicated that the specific ratios of solvents within the extraction mixture play a crucial role in the recovery of bioactive compounds, with a binary solvent system combining methanol and water proving to be the most effective for the compounds analyzed in *Centella asiatica* [20]. Akullo *et al.,* (2023) investigated the phytochemical composition and antioxidant properties of various solvent extracts from two distinct varieties of ginger and garlic and revealed that the free radical scavenging capacity of these extracts was influenced by their concentration levels. A significant positive correlation was observed between antioxidant activity and the total content of phenolic compounds and flavonoids. Notably, the ethanol extracts of local ginger demonstrated the highest antioxidant activity among the tested samples. The difference in ethanolic and methanolic extract antioxidant activity in different published papers related to the phytochemical compound variation in different plants [21]. Phenolic compounds of medicinal plants create one of the major groups of molecules acting as antioxidants [22]. The differences observed in flavonoid concentrations may stem from the inherent variability present in plant materials. A high concentration of flavonoids indicates the potential value of the plant, as these compounds play a crucial role in numerous nutraceutical, pharmacological, therapeutic, and cosmetic fields. Furthermore, flavonoids are recognized for their remarkable ability to neutralize a variety of oxidizing agents, such as singlet oxygen and several free radicals. The antioxidant effectiveness of flavonoids is influenced by the specific functional groups present and their spatial arrangement within the molecular framework. The results from this work demonstrated higher radical scavenging activity for methanol extract and moderate antimicrobial properties. It indicated that the results of this study could serve as basic scientific data information for the possible application of *J. stricticaulis* as a source of natural antioxidant and antimicrobial agents for future application.

Antifungal Activity

Inhibition zones of *J. stricticaulis* extracts are shown in Table 2. The results confirmed that in both growth stages, all extracts were more effective in Gram-positive bacteria than gram-negative bacteria and fungi. In the PF stage, diameter of the inhibition zone for the *S. aureus* was higher than the fungi. The Gram-negative bacteria were not affected by the extracts. The methanol extract had the least inhibitory effect. During the AF stage, the dichloromethane extract exhibited the most significant antibacterial activity against Staphylococcus aureus, whereas it demonstrated no effect on Escherichia coli. Conversely, the methanol extract produced the largest inhibitory zone against E. coli. Overall, the AF extracts displayed greater inhibitory efficacy compared to the PF extract across all tested micro-organisms. The variation in the sensitivity to *J. stricticaulis* extracts could be attributed to bioactive compounds especially phenolic compounds which are known as antimicrobial [23]. Bioactive compounds of

medicinal plants change during developmental stages [24,25]. In sum, ethyl acetate extract was found to be the most effective, followed by dichloromethane and methanol extracts. *E. coli* was resistant to extracts (Table 2). Various researchers already shown that Gram-positive bacteria are more sensitive to medicinal plants extract compared to Gram-negative bacteria. This various behaviors of the plant extracts may be due to several factors such as the permeability barrier induced by outer membranes in Gram-negative bacteria or the special enzyme activity of periplasmic space that can break down molecules that enter from outside [26]. Antimicrobial activity of medicinal plants related to phenolic constituents [27]. There are a few reports about the secondary metabolites of *J. stricticaulis* although some researchers in different species of Johreniopsis evaluated essential oils constituents. The secondary metabolites profiles of medicinal plants are affected by growth stage and in most medicinal plants at the flowering stage bioactive compounds are higher than the plants at before flowering stage. Azizi (2008) showed that in *Hypericum perforatum* the best harvest time for extracting higher amount of oil is the full flowering stage [28, 29]. Other studies showed that Gram-positive bacteria are more sensitive than Gram-negative bacteria [30]. Higher resistance of Gram-negative bacteria to external agents is attributed to the presence of lipopolysaccharides in their outer membranes, which make them inherently resistant to antibiotics, detergents and hydrophilic dyes [31].

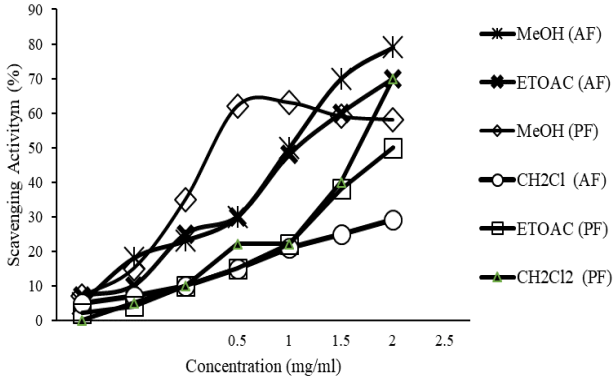

Fig. 1 Scavenging activity of different *J. stricticaulis* extracts in two stages (AF: after flowering stage, PF: Pre flowering stage).

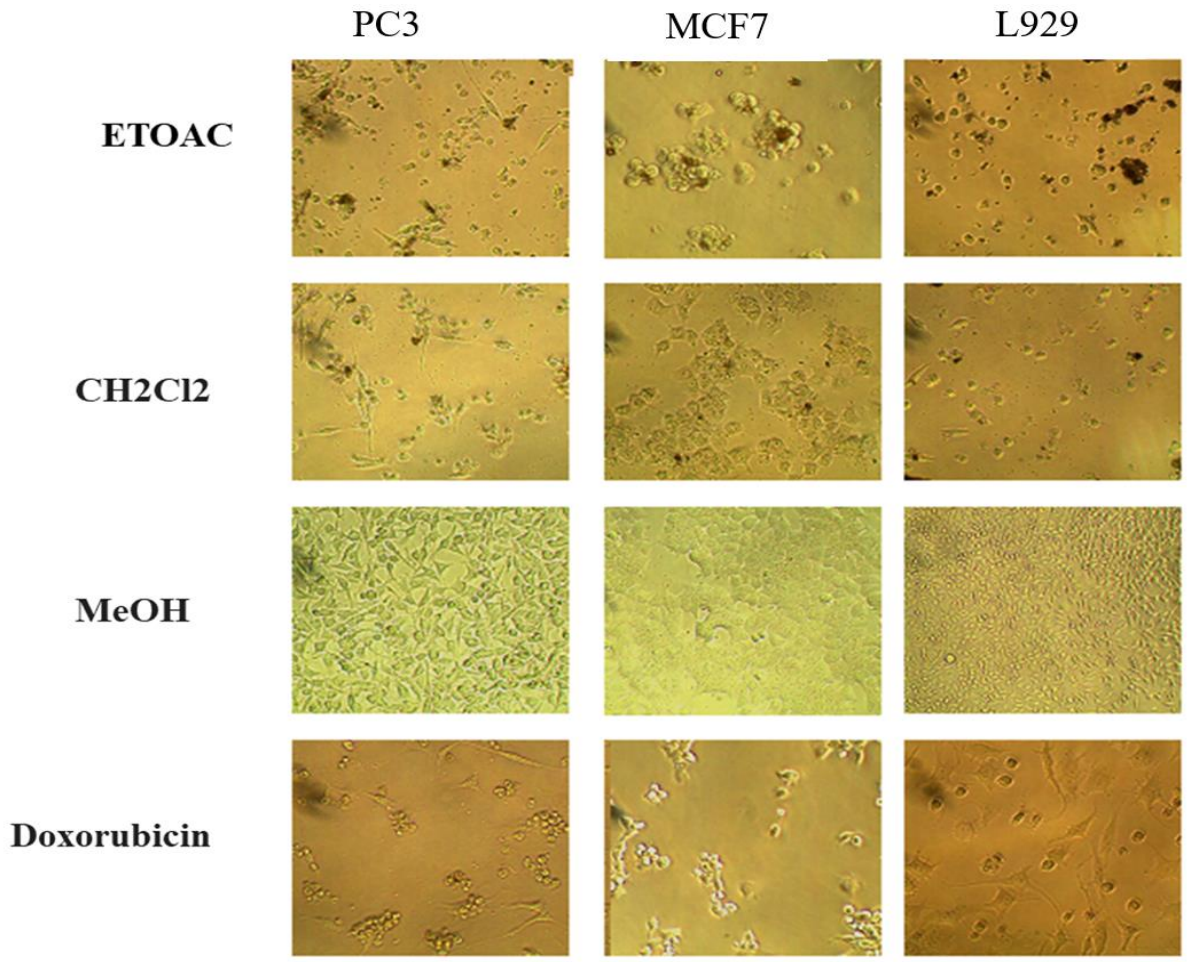

Fig. 2 Microscopic view of the treated cells with 100 µg/ml concentration of the extracts

Table 2 Diameter of inhibition zones of *J. stricticaulis* extracts, positive and negative controls in Disk and well-diffusion methods.

Sample	Diameter of inhibition zones [a] (mm)					
	S. aureus		*E. coli*		*C. albicans*	
	Disk	Well	Disk	Well	Disk	Well
MeOH (PFstage)	11.0 ± 0.2	12.0 ± 0.24	6.0 ± 0.61	6.0 ± 0.54	8.0 ± 0.19	8.66 ± 0.48
ETOAC (PF stage)	14 ± 0.15	16.66 ± 0.32	6.0 ± 0.27	6.0 ± 0.65	13.3 ± 0.16	9.33 ± 0.16
CH_2Cl_2 (PF stage)	12.66 ± 0.41	14.66 ± 0.52	6.0 ± 0.38	6.0 ± 0.36	12 ± 0.32	12.33 ± 0.34
MeOH (AF stage)	19.0 ± 0.62	15.33 ± 0.34	14.0 ± 0.29	13 ± 0.59	14.33 ± 0.48	11.66 ± 0.52
ETOAC (AF stage)	16.33 ± 0.57	15.0 ± 0.28	6.0 ± 0.12	6.0 ± 0.17	12.33 ± 0.45	11.33 ± 0.35
CH_2Cl_2 (AF stage)	22.33 ± 0.45	16.33 ± 0.51	13.33 ± 0.46	11.33 ± 0.18	15 ± 0.36	12.3 3± 0.24
Gentamycin	39.66 ± 0.14	40 ± 0.57	38.0 ± 0.26	30.0 ± 0.69	NT	NT
Nystatin	NT	NT	NT	NT	30±0.51	28.0±0.72
DMSO b	-	-	-	-	-	-

[a] Expressed as the size of the inhibition zones (mm) as an average of triplicates

[b]DMSO as negative control had no antibacterial activity. NT means not tested.

Table 3 Cytotoxic activity of *J. stricticaulis* extracts against cancer cell lines by MTT assay (µg/ml).

IC_{50}(µg/ml) Cells	Time	ETOAC PF stage *	ETOAC AF stage **	CH_2Cl_2 PF stage	CH_2Cl_2 AF stage	MeOH PF stage	MeOH AF stage	Doxorubicin
PC3	24h	>300	175.0 ± 2.9	>300	155.0 ± 3.4	>300	>300	1.05 ± 0.03
	48h	156 ± 2.1	85.4 ± 1.1	242.5 ± 5.4	91.7 ± 2.4	>300	239.5± 4.7	0.18 ± 0.07
	72h	165.3 ± 2.5	85.9 ± 1.5	251.7 ± 7.3	95.9 ± 2.3	>300	244 ± 4.5	0.30 ± 0.06
MCF7	24h	>300	190.7 ± 3.1	>300	157.7 ± 4.4	>300	>300	1.34 ± 0.03
	48h	202 ± 2.3	80.7±2.4	282 ± 3.3	82.4 ± 1.8	>300	246.3 ± 5.5	0.25 ± 0.02
	72h	210.3 ± 2.6	75.4±2.2	292.3 ± 4.8	75.0 ± 1.6	>300	234.1 ± 6.1	0.10 ± 0.03
L929	24h	>300	158.3 ± 4.4	>300	135.0 ± 4.7	>300	>300	1.88 ± 0.03
	48h	221 ± 8.6	81.6 ± 2.6	307 ± 6.3	86.0 ± 2.7	>300	226.8 ± 5.8	0.88 ± 0.06
	72h	198.3 ±7.9	79.1± 2.1	298 ± 6.2	85.7 ± 2.3	>300	236.4 ± 6.7	0.70 ± 0.05

*Pre flowering stage, *After flowering stage, Data are mean ±SD values

Also, Gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier [32]. In MIC-MBC assay there weren't substantial differences between the MICs of various extracts. All of the extracts had antibacterial activity just in high concentrations. Some researcher presented the mechanism of antibacterial activity of plant metabolites. It has been proposed that essential oil (EO) constituents penetrate the cellular membrane and inflict damage on organelles without altering the membrane's structure. Research also indicates that EO inhibits receptors that are responsible for detecting signals from various autoinducers and exhibits antibacterial properties.

Furthermore, studies have demonstrated that 1,8-cineole induces alterations in the morphology and dimensions of both Gram-negative and Gram-positive bacterial cells. Observations revealed that exposure to EO resulted in significant condensation of nuclear chromatin within the nucleoplasm, ultimately triggering apoptotic processes in the affected cells [33]. The antibacterial properties may be attributed, at least in part, to a disruption of the lipid bilayer of the cytoplasmic membrane, which facilitates the leakage of essential intracellular components. The researchers proposed that the efficacy of the antibacterial action is influenced by the lipid composition and the charge present on the surface of the bacterial membrane. Notably, the presence of lipopolysaccharides on the plasma membrane of Gram-negative bacteria appears to account for the resistance exhibited by these bacteria against highly hydrophobic substances, as these lipopolysaccharides restrict the ability of such compounds to penetrate the membrane effectively [34].

Cytotoxicity Activity

The cytotoxic activity of *J. stricticaulis* extracts against three human tumor cell lines PC3 MCF7 and L929 cells were investigated using various concentrations ranging from 12.5 to 200 µg/ml by MTT test. The data showed that the tested extracts induced a significant decrease in human cell growth in a dose-dependent manner. The variation in cytotoxic activity of extracts could be attributed to

the nature of their bioactive compounds. The results of the *J. stricticaulis* plant extract effect on three cell lines PC3, MCF7 and L929 on PF with three extraction methods using solvents with different polarities such as methanol, dichloromethane and ethyl acetate and the essential oil of this plant have been reported in Table 3. The IC_{50} of each extract was obtained based on the MTT assay at 24, 48 and 72h time intervals. The concentration is based on µg/ml. Doxorubicin was tested as a positive control. Data from three separate experiments are reported as mean ± standard deviation. As the most noticeable changes in the results of the extract effects were observed after 48h, the statistical analysis was performed for the data obtained from this time. The amount of effective concentration of methanol extract is over-defined in all three cell lines; therefore, the methanol extract was removed due to the lack of influence on the process of cell growth changes. Accordingly, as shown in the following Table 3, there was no significant difference in the effect of two extracts of ethyl acetate and dichloromethane of *J. stricticaulis* on the first round of plant collection on two types of PC3 and MCF7 cancer cells compared to the normal L929 cells ($P\leq 0.05$). However, no significant difference was observed in the level of growth inhibition between the cancer and normal cells. The results of the effect of *J. stricticaulis* plant extract on three cell lines PC3, MCF7 and L929 on the AF with three extraction methods using solvents with different polarities such as methanol, dichloromethane and ethyl acetate has been reported in the Table 3. Data from three separate experiments are reported as mean ± standard deviation. As the most obvious changes in the results of the extract effects were observed after 72 h, the statistical analysis was performed for the data obtained from this time. After analyzing the data, using ANOVA test, no significant difference was observed in the effect of ethylene acetate, dichloromethane and methanol extracts of *J. stricticaulis* on the second round of plant collection on two types of PC3 and MCF7 cancer cells compared to the normal L929 cells ($P\leq 0.05$) (Fig 2). Also, the effect of these three extraction methods form *J. stricticaulis* plant using solvents with different polarities such as dichloromethane, ethyl acetate and methanol was compared in the second round of plant collection on the mentioned cell lines. Examining these data in Bonferoni test, the effect of *J. stricticaulis* methanol extract was lower on cell death than the other two methods ($P\leq 0.05$). There are not any reports about *J. stricticaulis* but some other research confirmed cytotoxicity of another medicinal plant extract [35, 36]. Mueed *et al.,* (2023) reported the different extracts of medicinal plants present different activities and the difference depend on the phytochemical of the extracts [37]. Previous studies with medicinal plants extract showed its potent anticancer effects on solid tumors, such as hepatocellular carcinoma, breast cancer, and lung cancer [36].

CONCLUSION AND FUTURE RESEARCH DIRECTIONS

The flowering process represents a distinctive phonological event in medicinal plants, characterized by synthesizing various secondary metabolites. The findings of this study indicate that to achieve enhanced biological activity from the extract of *J. stricticaulis*, it is advisable to harvest the plant post-flowering. Future investigations will focus on identifying the primary phytochemical constituents that contribute to the beneficial properties of *J. stricticaulis.*

In recent years, there has been a notable revival of interest in using medicinal plants and herbal remedies. The significance of these plants in addressing various health conditions has led to their overexploitation, resulting in the decline and potential extinction of numerous species in their natural environments. Despite their therapeutic potential, herbal medicines face several challenges, including issues related to low solubility, stability, and bioavailability. Additionally, some herbal formulations are susceptible to physical and chemical degradation, which can diminish their pharmacological effectiveness. In the past few decades, the application of nanotechnology in the development of herbal drug formulations has garnered considerable attention, primarily due to its ability to enhance therapeutic efficacy and address the limitations associated with traditional herbal medicines. Looking ahead, the implementation of nanotechnology-based delivery systems that are biocompatible, biodegradable, and derived from lipids, polymers, or nanoemulsions holds promise for improving the solubility, stability,

bioavailability, and overall pharmacological activity of herbal products.

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