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The cytotoxicity effect of crude aqueous and alcoholic extracts of Juncus rigidus on human lung cancer cell line (A549) in vitro

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***Abstract*---**The current study dealt with the identification on cytotoxicity effective of crude water and alcohol extracts from the Juncus rigidus on human lung cancer cells A549. The quantitative estimation of the active compounds of the water and alcohol extracts of the Juncus (Phenols - Flavonoids - Alkaloids - Tannins) was calculated The results of the aqueous extract were (8.82,5.91,13.42,16.29) mg/g, respectively. While the results of the crude alcoholic extract of the plant were (7.01,6.06,13.84,16.34) mg/g, respectively. The cytotoxicity effect of the water and alcohol extracts of the plant was tested on human lung cancer cells A549 and took six concentrations (200,100,50,25,12.5,6.25) micrograms /millilters for 3 exposing times (72, 48, 24) Hr, which recorded highest inhibition rate was for the alcoholic extract of the plant at a concentration (200) µg/ml, for an exposure period (72) hours, and for a probability level (0.05) that reached (83.45%), while (IR) of cancer cells obtained from the aqueous extract was recorded (68.96%).

***Keywords*---**Juncus rigidus, cytotoxcity, inihibition Rate (IR), A549.

Introduction

Cancer is one of the most important causes of death in the world if its cells are characterized by unlimited cell growth and division, and the person remains at risk of infection throughout his life despite the improvement in survival rates, according to the World Health Organization and the IARC for the year 2020 record (19.3) new infections and (10000000) dead around the world, the most common cancer among which was breast cancer among women, and it amounted to (2.29) million deaths, while (2.21) million deaths were recorded among men with lung cancer [1]. Although there are many methods of treatment for cancer, such as chemotherapy and physical therapy, in addition to genetic therapies and surgical

intervention, it represents a problem and a challenge for scientists, so the shift was made towards searching for therapeutic alternatives to replace the treatments offered now, which prompted the adoption of a new approach in the methods of searching for medicines And new compounds with lower cost and toxicity, if current treatments are expensive and affect healthy cells as well. Several anti-cancer compounds of natural origin were found as by-products of metabolism in plants that target cancer cells and have little toxicity to normal cells [2].

Juncus rigidus is a perennial herbaceous plant belonging to the family Juncaceae, found in Asia and Africa as well as the southern hemisphere. It is considered a medicinal plant if used in traditional medicine as a diuretic and treatment of stomach ulcers and diarrhea. *Juncus rigidus* contains many compounds Secondary metabolism with vital activity such as phenolic acids, alkaloids, flavonoid compounds and tannins, which are natural antioxidants and anti-cancer [3]. The aqueous and alcoholic extracts of *Juncus rigidus* contain antioxidants with great efficacy in removing free radicals and anti-cancer. Several studies have indicated that the extracts of the vegetative group of Juncus contain many compounds of medicinal use. They are used in the treatment of muscle spasms and diseases of the Circulation and the nervous system, in addition to the presence of compounds that have a high efficacy to kill cancer cells, such as phenanthrene and its derivatives, as the ability of these compounds of the tuberculosis plant and its effectiveness in inhibiting cancer cells in human cancerous cell lines such as lines MCF7 and A549 in addition to other lines [4].

Materials and Methods

The plant was collected from the banks of the Al-Musayab project river in Babylon Governorate in Iraq for the month of September 2021. The vegetative sum (stalks and leaves) of the plant was taken, then washed with water and left in the shade until drought. The aqueous and alcoholic extract was prepared according to the method [5] where 50 g of vegetable powder was weighed and added to 500 ml of distilled water and then placed on the magnetic stirrer for three days at a temperature of (23-28) °C, then the mixture was filtered with a clean cloth after that by paper Filter (Whitman No1) and pour into plastic dishes to be dried in the incubator at a temperature of 38 °C. The alcoholic extract was prepared in the same way as before, but using ethyl alcohol at a concentration of 70%.

Quantitative determination of the active compounds in the water and alcohol extracts of the vegetative group (stems and leaves) of *Juncur Rigidus*

- The phenols were determined using a method [6].
- The quantification of Taninns content of dry plant extracts was calculated by [7].
- The flavonoids have been estimated by following [8].
- Alkaloid compounds were estimated using [9].

Cytotoxicity assay

The aqueous and alcoholic extracts were prepared using the method [10]. A weight of 0.1 g of the extract was dissolved, then it was filtered using filter paper with holes (0.45 - 0.22) micron, and the concentrations were (200,100,50,25,12.5,6.25) .Then preparing the suspended cells of the cancer line (A549) using trypsin-versene solution after adding 2ml of it after washing the old medium with phosphate buffer solution (PBS), then adding it to the tissue culture bottle (50 cm^3) and then adding 20 ml of medium containing bovine blood serum 10% and mix well. Then 0.2 ml of cell suspension was taken and transferred to the pits of the tissue calibration dish containing 96 holes using an automatic pipette. Then the dishes were incubated at a temperature of 37 °C until the cells adhered to the bottom of the pits. Then they were washed with PBS solution and 0.1 ml was added to each hole of The MTT stain was then washed with buffer solution to get rid of the excess dye after an incubation period (3 hours), then dimethyl sulphoxide (DMSO) solution was added to each hole and incubated for 15 minutes [10]. The results were read using an ELISA device with a wavelength of 492 nm. The inhibition ratio. And by means equation, it was calculate IR:

$$(IR) = A- B/A*100$$

IR= the percentage of inhibition rate.
A= the optical density of the control treatment
B= the optical density of the samples[11].

Results

Results of quantitative determination of the active compounds of the vegetative group (stems and leaves) of *Juncus rigidus*

The results showed that the quantification of the phenolic compounds of the aqueous extract (16.29 mg/g) and the alcoholic extract (16.34 mg/g), respectively, while the quantitative determination of the tannins in the aqueous extract was (8.82 mg/g), while the alcoholic extract was (7.91). The results of the quantification of the alkaloid compound were recorded for the aqueous extract (5.91 mg/g) and the alcoholic extract (6.06 mg/g). As for the flavonoids, the results of the quantification of the alkaloid compound were recorded for the aqueous extract (13.42 mg/g) and the alcoholic extract (13.84mg/g). As shown in Table (1).

Table 1
Quantitative determination of the active compounds of the water and alcohol extract of the(stems and leaves) of *Juncus rigidus*

N	Active Ingredients / Extracts	Phenols	Tannins	Alkaloids	Flavonoids
1	Aqueous extract mg/g	16.29	8.82	5.91	13.42
2	Alcoholic extract mg/g	16.34	7.91	6.06	13.84

Effect toxic of crude aqueous extracts of vegetative group of *J. rigidus* on human lung cancer line A549 for 3 Times (24,48,72) Hr

The results shown in Figure (1) showed that the effect of the aqueous extract and the effectiveness of killing varies depending on the concentration of the used and the exposure period. The lowest percentage of cancer cell inhibition was obtained at the concentration (6.25µg/ml), while the highest percentage of cancer cell killing was at The concentration (200) micrograms/millilters for 3 exposing times (24,48,72)Hr were (68.96±2.15, 54.12±2.11, 37.51±1.75) respectively compared to the other concentrations with a significant difference at the probability level ($P \leq 0.05$). While the lowest inhibition of human lung cancer cells was obtained at the concentration (6.25 µg/ml) was (10.13±.031) for the same probability level after 24 hours.

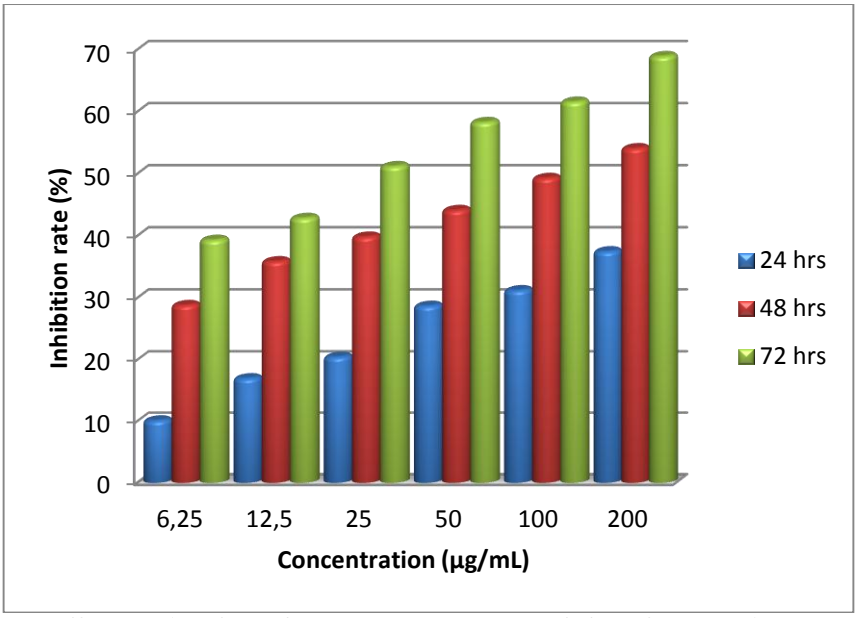

Figure 1. Effect toxic of crude aqueous extracts of the of vegetative group 0f *J. rigidus* on human lung cancer cell line A549

Toxic effect of crude alcoholic extracts of vegetative group of *J. rigidus* on human lung cancer line A549 for 3 Times (24,48,72)Hr

A results showed in Figure (2) that the toxic effects of alcoholic extract on human lung cancer line A549 cells began to appear at the lowest concentration (6.25 $\mu g/ml$) and for 3 exposure times (72,48,24) hours, where the percentage of inhibition was (42.27%±1.12), (30.64%±5.26), (15.29%±3.11) respectively at the probability (0.05), where the toxic effect appeared at the lowest concentration with a high percentage of inhibition with increasing concentration and exposure time, reaching (83.45%) at concentration (200 µg/ml). of the extract at the probability level (0.05) within 72 hours of treatment.

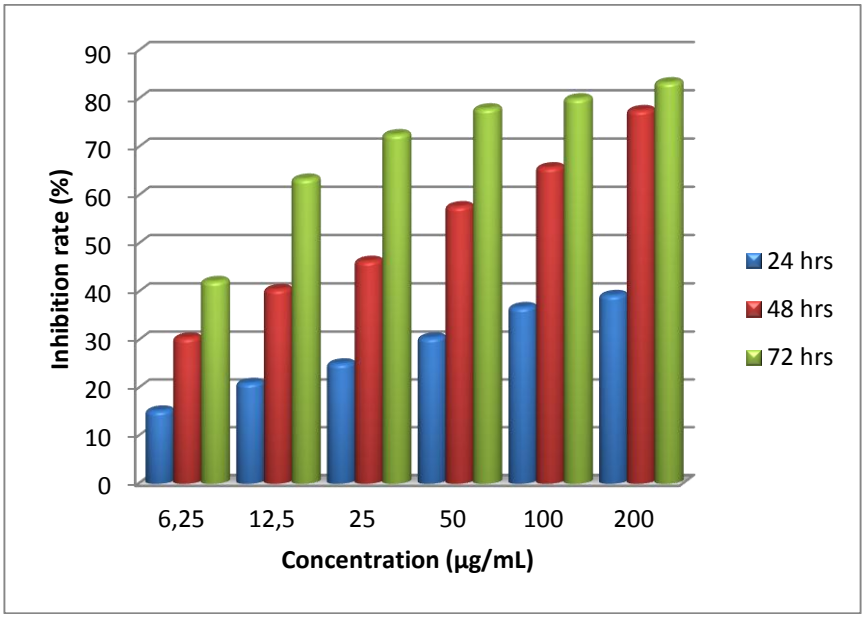

Figure 2. Toxic effect of the crude alcoholic extract of vegetative group (stems and leaves) of *J. rigidus* on Human lung cancer line A549

Comparison of toxic effect of crude water and alcohol extracts of vegetative group of *J. rigidus* on human lung cancer line A549

The results shown in Figures (5,4,3) show that the alcoholic extract outperformed the aqueous extract in influencing cancer cells for all concentrations and periods of exposure to the extract. As we note in Figure (3) the toxic effect of the alcoholic extract shows a greater killing rate, especially in the high concentration, where it reached The percentage of inhibition of the alcoholic extract (39.38%±4.13) at the probability level (P≤ 0.05) and after 24 hours of the dose and at a concentration of (200µg/mg), while the aqueous extract gave the percentage of inhibition (37.51%±1.7) for the same concentration and exposure period at the probability level. It self.

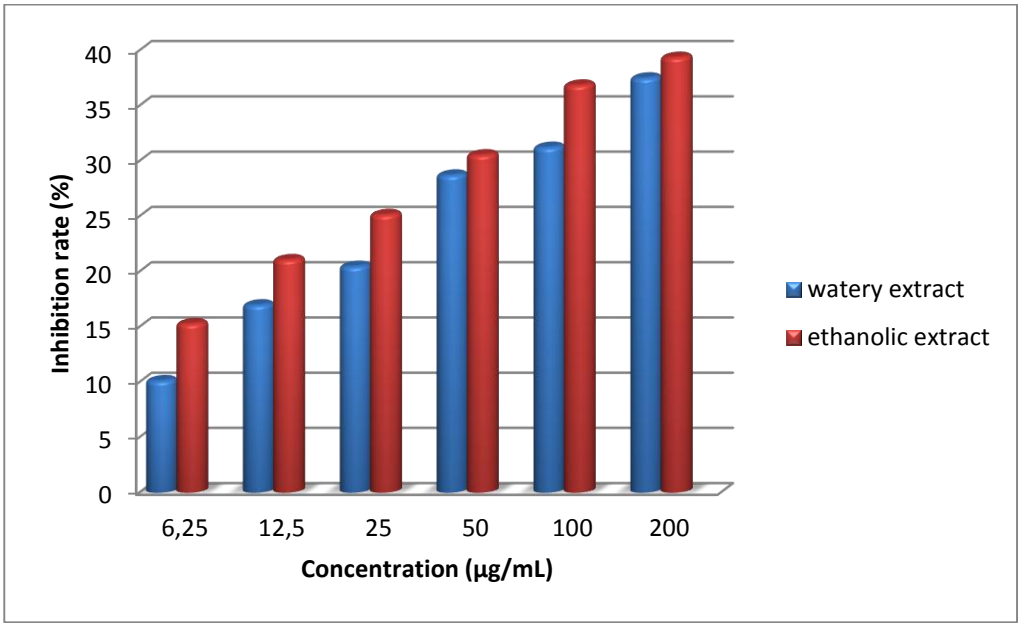

Figure 3. Comparison between the toxic effect of crude water and alcohol extracts of vegetative group of *J. rigidus* on human lung cancer line A549 after 24 hours

After (48) hours of treatment with the extracts, we find that the alcoholic extract has the predominant effect on the aqueous extract and for all concentrations, as the percentage of killing increases by increasing the concentration of both extracts, but there is a clear preference for the alcoholic extract, as the percentage of inhibition of cancer cells was (77.69%±5.11). For the highest concentrations (200 µg/ml), while the inhibition rate for the aqueous extract after 48 hours of exposure was ($54.12\% \pm 2.41$) at the same concentration used with a significant difference (23.53%) for the alcoholic extract at the probability level ($P \leq 0.05$). in Figure (4).

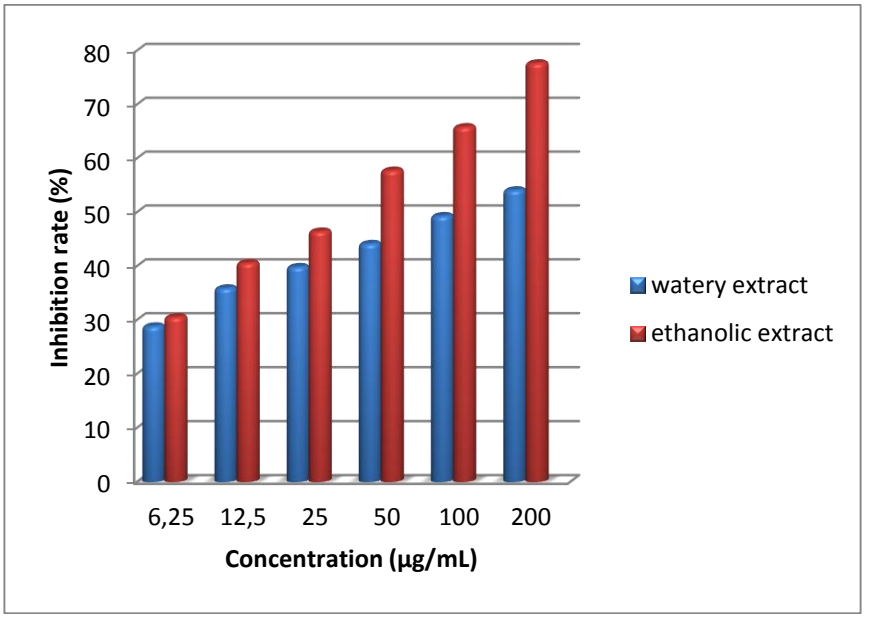

Figure 4. Comparison between toxic effect of the crude aqueous and alcoholic extract of vegetative group (stems and leaves) of *J. rigidus* on human lung cancer line A549 after 48 hours

Likewise, the situation did not change after 72 hours of exposing the cells of the A549 line to the aqueous and alcoholic extract of the plant, as the percentage of inhibition increased directly with the increase in the concentration used and for both extracts, where the highest value of inhibition was recorded for the alcoholic extract, reaching (83.45%) at the concentration (200) µg/ml .as shown in Figure (5).

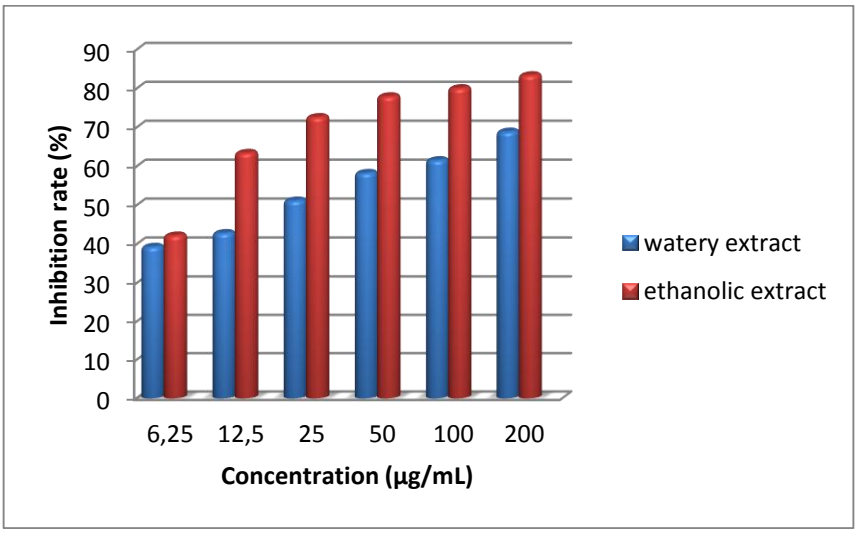

Figure 5. Comparison between the toxic effect of the crude wter and alcohol extracts of of vegetative group (stems and leaves) of plant rigidus. J on human lung cancer A549 line after 72 hours

Discussion

In recent years, the use of plant extracts has become common in medicine and pharmacological research because of their preventive and curative role, in addition to the emergence of the problem of therapeutic resistance due to the excessive use of chemical drugs and for many diseases, so it was necessary to search for natural alternatives, especially in medicinal plants, among those plants used *Juncus* plant, which contains a lot of biologically active compounds and antioxidants, where the quantitative estimation results of aqueous and alcoholic extracts showed that the plant contains good amounts of compounds with biological activities and anti-cancers such as phenolic acids and tannins that have proven their effectiveness as natural antioxidants in addition to the presence of flavonoids that work on Elimination of free radicals formed in the body from activities, and the *Juncus* genus is famous for its possession of the phenanthrene compound, which has a high activity against cancer [12].

The study [13] indicated that the water and alcohol extracts of Juncus plant contain chemical compounds with anti -inflammatory activity, in addition to the presence of the compound Dehydroffusol, which has toxic properties for cancer cells, including human lung cancer cells A549, as it works to stop the growth and destruction of cells and prevent Spread of malignant cells to other tissues by activating the programmed cell death mechanism. Some studies, including the study [14], indicated the effectiveness of water and alcohol extracts of the *Juncus* in inhibiting the growth and spread of lung cancer cells in a manner that depends on the type of extract and the sensitivity of the cells towards it, in addition to the concentration used and the duration of exposure, which led to activating the mechanism of cell death and causing damage to the body. Genetic material in malignant cells and preventing the expression of genes responsible for the formation of blood vessels that nourish the cancerous tissue and preventing glucose metabolism within the mitochondria of cancer cells and thus cell death.

There are many studies, including the study [15] of the important role of medicinal plants, and among those plants is the genus Juncus, which possesses many compounds with biological activity against many diseases in addition to antioxidants, and many secondary metabolites such as hydrophenanthrin compounds and flavones and Apigenin and luteolin, which show strong toxic activity on triple negative human breast cancer cells (MDA-MB231) by stopping the growth and spread of cancer cells to healthy tissues. A recent study [16] indicated that plants of the Juncaceae family contain many derivatives of natural chemical compounds that are characterized by their role as anti-inflammatory and anti-cancer, in addition to their high scavenging effectiveness of free radicals that damage the genetic material inside the cell, thus working to prevent cancer from By stopping the cell cycle and preventing uncontrolled reproduction. The halal medicinal plants, including the genus Juncus, are of great importance in alternative medicine, as they have many active molecules that are used to make treatments that replace the traditional medicines that exist at the present time. *Juncus* is one of the most promising plants as natural sources for the treatment of many diseases due to its increased content And the variable is one of the compounds that promote human health [17].

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