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Cytotoxic effect of the crude alcoholic extract of Juncus rigidus on cells of the human breast cancer line MCF-7 in vitro

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***Abstract*---**This study tested the toxicological activity of the crude alcoholic extract of the vegetative part of Juncus rigidus on cells of the MCF-7. The quantitative estimation of the active compounds of the alcoholic extract (alkaloids - tannins - flavonoids - phenols) was found, as the results gave (6.06 - 7.91 - 13.84 - 16.34 mg/g) respectively. The antioxidant activity of the alcoholic extract was also calculated by DPPH method. The percentages of free radical inhibition were (9.31%, 16.43%, 19.81%,20.51%,24.31%) at concentrations (10,20,30,40,50 mg/ml) respectively. Six concentrations of (6.25, 12.5, 25, 50, 100, 200 µg/ml) of alcoholic extract of the vegetative part of the plant were tested on the growth of human breast cancer cells MCF-7 line for (3) exposure periods (24,48,72) Hr .Where the extract had a toxic effect on cell growth from the lowest used concentration (6.25 µg/ml), where the inhibition percentage was recorded (2±32.8%) for the 72-hour exposure period and for a probability level (P≤ 0.05) versus (72.3%±2) at the concentration (200 µg/ml) and for the same exposure time and probability level.

***Keywords*---**Juncus rigidus, Cytotoxcity, DPPH, MCF-7.

Introduction

The term cancer refers to any disease characterized by the development of abnormal cells that divide abnormally out of control and have the ability to infiltrate and destroy other normal tissues. Survival rates from cancer vary greatly depending on the type of cancer, the age of the patient and the general health status of the affected person, and the stage of the disease, the earlier the

diagnosis, the greater the survival rate and chance of survival. According to the report issued by the (WHO) on March 3, 2021, cancer is one of the main causes of death, as statistics for the year 2020 showed that it claimed the lives of more than 15 million people, and one of the most prominent types of lung cancer was (2.21) million deaths among In men and women, breast cancer has emerged, causing (2.26) million deaths [1].

The family Juncaceae, to which the genus *Juncus* belongs, spreads all over the world if it is found in Asia, Africa and South America. *Juncus rigidus* [2]. The plant is characterized by possessing many secondary metabolic compounds that have activity against many diseases such as diseases of the digestive system and circulatory system. It also acts as a diuretic and anti-inflammatory. Also, the alcoholic extract of the vegetative part (the stem and leaves) of the rush plant contains many compounds. Effective alkaloids, flavonoids and phenolic acids, which act as natural antioxidants, as well as the active substances of the tuberculosis plant remove harmful free radicals, and among the most important compounds are phenanthrens, apigenin and gucosides that have an anti-growth effect on different types of cancerous lines and the human breast cancer line MCF- 7 [3].

Many of the medicines and treatments used today against cancerous diseases contain a lot of risks due to the side effects resulting from the treatment itself, so there was a need to search for new materials that have fewer side effects and a high curative effect. Fortunately, these compounds were found in nature, especially Medicinal plants, including the genus *Juncus*, whose aqueous and alcoholic extracts contain many chemical compounds that have an important role in fighting cancer in addition to their role in regulating some physiological activities in the body. Breast cancer incidence. Secondary metabolites such as alkaloids, flavonoids, and tannins work to fight ROS, prevent inflammation, reduce or prevent cell damage caused by free radicals, and boost immunity[4]. There are many compounds that were isolated from the extracts of the rush of *Juncus* plant, including Dehydroffusol, which is effective against the vascular cells of stomach cancer and prevents the consistency of cancer cells by reducing the protein responsible for this process on the surface of cells, where it prevents the migration and spread of cells, as revealed by a study Molecular Mechanisms Dehydroffusol effectively inhibited the expression of the major angiogenesis gene VE-Cadherin in gastric cancer [5].

Materials and Methods

Plant collection and extract preparation

The plant was collected from the local environment in central Iraq from the banks of the rivers in the fall of 2021, the vegetative sum (the stem and leaves) of the plant was taken and washed well to remove the suspended soil and dried in the shade away from direct sunlight and grinded using the electric grinder and the powder was kept in tightly closed plastic containers. The alcoholic extract was prepared by following the method [6] where 50 g of powder was weighed and then placed in a glass container and half a liter of 70% ethyl alcohol was added and left on the magnetic stirrer for 72 hours, then filtered using gauze and then by paper

(Whitman No1) And dried in the incubator after pouring into plastic containers at a temperature of 40 °C.

Quantitative determination of the active compounds in the alcoholic extract of the vegetative part (stem and leaves) of the plant *J. Rigidus*

- The total content of flavonoids was estimated using the method used in [7].
- The total content of the alkaloid compounds was estimated according to the method [8].
- The phenols were determined using a method [9].
- The method [10] was taken to estimate the total content of tannins.

Antioxidant efficacy of alcoholic extract free radicals

The antioxidant activity of free radicals was measured using the DPPH method, based on [11].

Toxic effect of alcoholic extract on cells of the human breast cancer line MCF-7

The MCF-7 human breast cancer cell line from the Iraqi Company for Biotechnology was prepared at passge 35 and the cells were grown on (RPMI1640) culture media by adding 10% fetal bovine blood serum, to which (100 µg) of streptomycin was added, then the cells were passed twice a week using (trypsin-EDTA) and incubated at 37°C. The alcoholic extract was prepared using the method [12]. Where 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 concentrations of (6.25, 12.5,25, 50,100,200 µg/ml) were banned .Then the suspension of human breast cancer cells (MCF-7) was prepared using a trypsin-peresene solution after adding 2ml of it after washing the old medium with phosphate buffer saline (PBS), then adding it to the tissue culture bottle of (50 cm^3) and then adding 20ml of medium containing 10% bovine serum 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 a micropipette. 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 phosphate buffer solution PBS and 0.2 ml of concentrations was added. The prepared extract was previously prepared and incubated at a temperature of 37°C until the end of the exposure period, then washed and 0.1% MTT stain was added to each hole, then washed with buffer solution to get rid of the excess dye after an incubation period (3 hours), then dimethyl sulfoxide solution was added to each hole. (DMSO) and incubated for 15 minutes [13]. The results were read using an ELISA device with a wavelength of 492 nm. The inhibition ratio was calculated using the equation:

$$\text{Inhibition rate} = A- B/A*100$$

Where 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 in the alcoholic extract of the the vegetative part (stem and leaves) of *Juncus rigidus*

The results of the study showed that the quantitative determination of the active compounds in the alcoholic extract of the vegetative part (stem and leaves) of *Juncus rigidus* as shown in Table (1).

Table 1
Results of the quantification of the active compounds in the alcoholic extract of the the vegetative part of *Juncus rigidus*

N	Active Ingredients / Extracts	Phenols	Tannins	Alkaloids	Flavonoids
1	Alcoholic extract mg/g	16.34	7.91	6.06	13.84

Antioxidant potency test

The results of the DPPH test showed the percentages of free radical inhibition of the alcoholic extract of the vegetative part (stem and leaves) of *Juncus rigidus*, as shown in Table (2).

Table 2
The percentages of free radical inhibition in the alcoholic extract of the vegetative part (stem and leaves) of *Juncus rigidus*

N	Extract Concentrations (mg/ml)	0	10	20	30	40	50
		DPPH inhibition %					
1	Alcoholic extract	0	9.31	16.43	19.81	20.51	24.31

Study of the cytotoxic effect of the crude alcoholic extract of the vegetative part (stem and leaves) of *Juncus rigidus* on cells of the human breast cancer line MCF-7 for the three exposure times (24, 48,72) hours

The results of the effect of alcoholic extract of the shoot (stem and leaves) of *Juncus rigidus* on the growth of MCF-7 human breast cancer cells, as shown in Figure (1), showed a cytotoxic effect on cancer cells depending on the concentration and duration of treatment with the extract. The results showed an increase in the percentage of For the rate of inhibition (IR) with increasing concentration, where the concentration (200 µg/ml) gave the highest inhibition value at 72Hr, reaching (72.33%±2.31), while after 48Hr of exposing MCF-7 cells to the alcoholic extract, the percentage of inhibition reached (54.5%±2.45) at a

concentration of (200 µg/ml), while the concentration (6.25 µg/ml) gave the lowest percentage of cell death, which amounted to (10%±2.34). While the results recorded low rates for exposure time of 24Hr for all concentrations, as the lowest rate of cell inhibition was (8.45%±2.05) at the concentration (6.25µg/ml), while the percentage of inhibition reached (31.82%±1.6) at concentrations (200 µg/ml).

Table 3
Shows the cytotoxic effect of different concentrations of the shoots (stems and leaves) of *Juncus rigidus* and for three periods (24, 48,72) Hr of exposure on human breast cancer cell line MCF-7

Time / Con	24	48	72
6.25	8.45±2.05Aa	10±2.34Aa	32.85±2.06Ab
12.5	15.57±1.94Ba	28.85±2.98Bb	51.17±2.28Bc
25	17.7±1.61BCa	34.06±3Cb	57.71±1.40Cc
50	19.67±3.3Ca	36.03±2.69Cb	60.40±0.38Cc
100	26.94±1.8Da	40.01±0.83Db	64.34±1.33Dc
200	31.82±1.63Ea	54.5±2.45Eb	72.33±2.31Ec
$LSD_{0.05}$+	3.44		

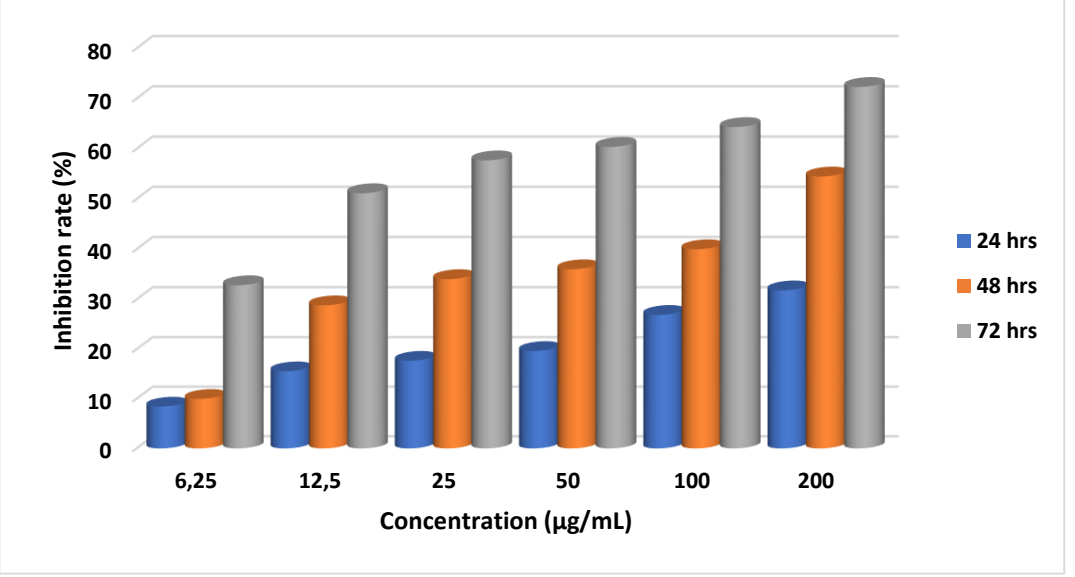
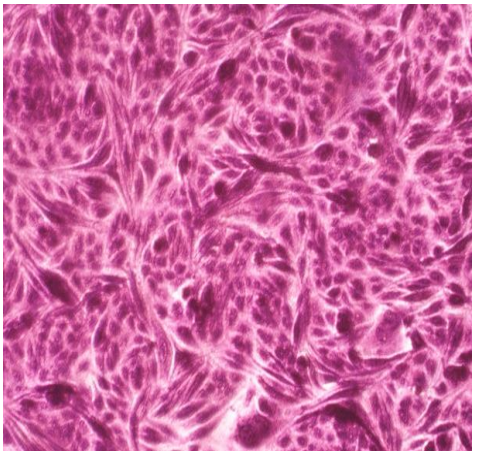
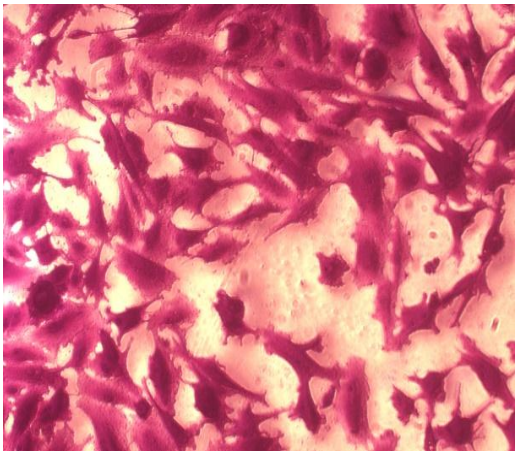


Figure 1. The cytotoxic effect of the crude alcoholic extract of the shoots (stems and leaves) of *Juncus rigidus* on cells of the human breast cancer line MCF-7 for triple exposure times (24, 48,72) hours



A



B

MCF-7 breast cancer cells not exposed to alcohol extract (control) (10x)

MCF-7 human breast cancer line cells exposed to the alcoholic extract after 72 hours (10x)

Discussion

Cancer has become a problem of the times and is one of the common diseases at the global level. The rate of injuries and deaths increases every year with various types of cancers, and this increase is steady, which prompted the trend to search for preventive and curative alternatives to replace the currently used, and the most important of these alternatives are natural compounds in plants, including *Juncus rigidus*, which is one of the medicinal plants available in the local natural environment, contains a group of compounds that show activities in many diseases, as it is used for the prevention and treatment of inflammatory diseases in addition to its role as an anti-cancer. It is a good combination of compounds with important biological activities and antioxidants, as well as positive effects to enhance the health of the body.

The study of natural compounds in the *Juncus* plant was conducted with the aim of discovering new anti-carcinogenic compounds and drugs that have low side effects indicated the presence of compounds with excitatory activities in the rush plant that have the ability to target some of the gene expression products of oncogenes such as the G-complex. quadruplex associated with the formation of cancer The study also showed that the compounds in the plant *Juncus* have the ability to bind with the grooves in the DNA sequences of the oncogene and prevent the expression of the same and thus prevent the formation of cancer .The results obtained confirmed that the alcoholic extract of the shoot of *Juncus rigidus* has a toxic effect on the human breast cancer cell line MCF-7 depending on the exposure period and the concentration of the extract, and this is what the study indicated [15] that the alcoholic extract of *Juncus rigidus* has cytotoxicity. On the cells of the MCF-7 line, it depends on the sensitivity of the cells towards the extract, in addition to the concentration used and the exposure time. The extract

stopped growth and cell division and prevented the spread of cancer cells at low concentrations. In high concentrations, it showed high toxicity and high killing rates by causing damage to the cells. The results of the DPPH test showed the antioxidant activity that the alcoholic extract of *Juncus* plant contains natural antioxidants, which had a role in eliminating harmful particles generated inside the cell and protecting healthy cells from oxidative stress as well as It also works to protect the genetic material from damage due to the activity of free radicals, and thus works indirectly to prevent the formation of Hebei cells. Third, among those compounds are flavonoids, alkaloids and phenolic compounds that work synergistically to scavenge reactive oxygen species ROS and thus protect healthy cells and prevent mutations that predispose to cancer. There is also another role that these compounds play in the prevention of inflammatory reactions and protection of epithelial tissue lining the body from bacterial infection. [16].

There are many studies that showed that the alcoholic extract of the *Juncus* plant possesses many compounds with anti-cancer activities, including the study [17] where it was shown that the alcoholic extract contains the compound Betulin, which belongs to the chemical family Tribes (which is abundant in the *Juncus* plant) has important pharmacological properties as Through its role in the anti-cancer activity and toxicity towards malignant cells, it also induces cells to enter into the process of programmed cell death and thus cell death, in light of the failure of traditional treatments, betulin acid compounds are promising experimental treatments that replace current drugs for cancer patients .The same study also showed the role of the aqueous extract of the *Juncus* plant in the prevention of infections, as the extract had a protective effect on the epithelial tissue lining the mouth by inhibiting the inflammatory reaction resulting from bacterial infection, as the same source indicated the role of the extract in the prevention of infections caused by some substances. Chemical exposure to the lining of the mouth.

References

1. Sekar, P., Ravitchandirane, R., Khanam, S., Muniraj, N., & Cassinadane, A. V. (2022). Novel molecules as the emerging trends in cancer treatment: an update. Medical Oncology, 39(2), 1-24.
2. Mohmoud S.A. AL-Mounasi & Fatin A-J- Mustafa.(2009). Protoscolicidal activity of aqueous and alcoholic extracts of Juncus rigidus (Junceceae) in vitro. Basrah Technical institutes, Bas.J.Vet.Res.Vol.8,No.1.
3. El-Shamy, A. I., Abdel-Razek, A. F., & Nassar, M. I. (2015). Phytochemical review of Juncus L. genus (Fam. Juncaceae). Arabian Journal of Chemistry, 8(5), 614-623.
4. Ma, W., Liu, F., Ding, Y. Y., Zhang, Y., & Li, N. (2015). Four new phenanthrenoid dimers from Juncus effusus L. with cytotoxic and anti-inflammatory activities. Fitoterapia, 105, 83-88.
5. Qi, J., Jiang, W., Chen, G., Zhou, D., & Li, N. (2020). Studies on the chemical components and biological activities of the genus of Juncus. Asian Journal of Traditional Medicines, 15(6).
6. Chadha, Y. (1976). The wealth of India. A dictionary of indian raw materials and industrial products, 11, 112-122.

7. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the university of chemical technology and metallurgy. 2005 Jul;40(3):255-60.
8. Ajanal M, Gundkalle MB, Nayak SU. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Ancient science of life. 2012 Apr;31(4):198.
9. Paśko P, Tyszka-Czochara M, Trojan S, Bobis-Wozowicz S, Zagrodzki P , Namieśnik J, Haruenkit R, Poovarodom S, Pinsirodom P, Gorinstein S . Glycolytic genes expression, proapoptotic potential in relation to the total content of bioactive compounds in durian fruits. Food Research International . 2019 Nov 1;125:108563.
10. Broadhurst RB, Jones WT. Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture. 1978 Sep;29(9):788 94.
11. Marinova G, Batchvarov V. Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulgarian Journal of Agricultural Science. 2011 Feb 1;17(1):11-24.
12. Mansoori, B., Mohammadi, A., Davudian, S., Shirjang, S., & Baradaran, B. (2017). The different mechanisms of cancer drug resistance: a brief review. Advanced pharmaceutical bulletin, 7(3), 339.
13. Su, X. H., Yuan, Z. P., Li, C. Y., Zhong, Y. J., Du, H. J., Wen, Y. Y., ... & Liang, B. (2013). Phenanthrenes from Juncus effusus. Planta medica, 79(15), 1447-1452.
14. Platella, C., Capasso, D., Riccardi, C., Musumeci, D., DellaGreca, M., & Montesarchio, D. (2021). Natural compounds from Juncus plants interacting with telomeric and oncogene G-quadruplex structures as potential anticancer agents. Organic & Biomolecular Chemistry, 19(45), 9953-9965.
15. Wada, A., Murakami, K., Ishikawa, Y., Amoh, T., Hirao, K., Hosokawa, Y., ... & Yumoto, H. (2022). Anti-Inflammatory and Protective Effects of Juncus effusus L. Water Extract on Oral Keratinocytes. BioMed Research International, 2022.
16. Hordyjewska, A., Ostapiuk, A., Horecka, A., & Kurzepa, J. (2019). Betulin and betulinic acid: Triterpenoids derivatives with a powerful biological potential. Phytochemistry Reviews, 18(3), 929-951.
17. Wada, A., Murakami, K., Ishikawa, Y., Amoh, T., Hirao, K., Hosokawa, Y., ... & Yumoto, H. (2022). Anti-Inflammatory and Protective Effects of Juncus effusus L. Water Extract on Oral Keratinocytes. *BioMed Research International*,2022.