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Evaluation of Antioxidant Activity and Effect of *Cyperus Rotundus* L Flower Extract on Prostate Cancer Cells

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ABSTRACT
Cyperus Rotundus, a member of the Cyperaceae family, is classified as a monocotyledonous weed. *C. rotundus* tuber has been recognized as a traditional remedy for indigestion, vomiting, diarrhea, and other intestinal ailments, particularly in children. It is light, bitter, astringent, sedative, carminative, diuretic, anthelmintic, expectorant, laxative and nerve tonic. This study presents an evaluation of the antioxidant activity of *Cyperus Rotundus* flower extract using the DPPH method for the first time. The analysis was conducted with two solvents: ethanol and dimethyl sulfoxide (DMSO). Additionally, the study assessed the mineral content with antioxidant properties through ICP-MS and the anticancer effects of the flower extract in the context of controlling and treating prostate cancer. The comparative analysis of the antioxidant activities of ethanol, dimethyl sulfoxide (DMSO), and vitamin C has demonstrated that vitamin C possesses a superior antioxidant capacity compared to the other solvents. Additionally, the extract obtained using DMSO as a solvent exhibits a higher antioxidant activity than that derived from ethanol solvent. IC50 comparison between two groups of extract with ethanol solvent and extract with DMSO solvent showed that the antioxidant activity of the extract depends on the concentration and solvent. Also, among the minerals with antioxidant properties, magnesium, and sulfur were the highest and the lowest for copper and selenium. Investigating the anticancer activity of flower extract between 24, 48, and 72 hours has shown that the lowest cell viability after 72 hours is at the concentration of 1600 µg/ml, and the flower extract as a new preventive or therapeutic drug for the treatment of patients to be considered prostate cancer and needs more research.
Keywords: Antioxidant activity, Ethanol, Dimethyl Sulfoxide, Minerals, Anticancer activity

INTRODUCTION
The World Health Organization has focused on developing traditional medicine since about 30 years ago to implement its slogan, "Health for all by the year 3000". This decision is based on two grounds, firstly, the lack of access of many people to essential healthcare services, which includes up to 80% of the population in some countries, and secondly, the lack of satisfaction with new medical treatments, especially regarding chronic diseases and the side effects of the chemical drugs. The World Health Organization approved using traditional medicine to prevent and treat diseases many years ago. In 1978, a statement was issued by the World Health Organization in the field of traditional medicine, which was modified in 2002 as follows: - "Traditional medicine is a general term that refers to both "traditional medicine systems such as Chinese traditional medicine, Indian Ayurveda and Greek-Arabic traditional medicine" and also "various local forms." Traditional medical treatments contain drug therapy (medicinal plants, animal, and mineral components) and non-drug methods (such as acupuncture, massage, and psychological treatments) [1,2]. Cyperus Rotundus is a member of the Cyperaceae family and is a monocotyledonous weed. One of the essential medicinal plants is Cyperus Rotundus, usually called Nagar Motha or Musta in India, and it is known globally as Suad Kufi or Nutsedge and belongs to the Cyperaceae family [3-5]. The parts consumed are tubers or bulbous roots. Cyperus Rotundus tuber is a home remedy for indigestion, vomiting, diarrhea, and other intestinal problems in children; it is light, bitter, astringent, sedative, carminative, diuretic, anthelmintic, expectorant, laxative, and nerve tonic. Cyperus Rotundus tuber decoction or soup is beneficial for diarrhea, dysentery, indigestion, vomiting, cholera, and fever. The underground stem of this plant is consumed as a diuretic, anthelmintic, windbreaker, and stimulant. The paste of dried underground stems with honey is given to children to treat dysentery and vomiting. The decoction of its underground stem is consumed to treat fever, tuberculosis, pneumonia, scabies, smallpox, and diarrhea [6]. Oxidative stress is one of the most prominent reasons for many chronic diseases caused by free radicals. The human body is constantly under the influence of oxidative stress. Oxidative stress occurs in the human body when free radicals overcome the body's antioxidant defense system, or in other words, the imbalance between cell destruction by free radicals and the body's antioxidant defense is called oxidative stress. Biological attacks on the organism or organisms of the body are usually referred to as oxidative stress [7-9]. The damage induced by oxidative stress leads to numerous types of diseases, including neurological diseases (Alzheimer's and Parkinson's, Huntington's, Autism), diabetes, atherosclerosis, rheumatoid arthritis, inflammation, kidney disorders, liver disorders, blood pressure, adult respiratory distress syndrome, aging, cataracts, obesity, vasculitis, glomerulonephritis, gastric ulcer, hemochromatosis and various types of cancers such as breast cancer [8,10]. The imbalance between the production of free radicals and antioxidants will give rise to the attack of free radicals on biological molecules. Free radicals (RSS, RNS, ROS) can target proteins, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules, carbohydrates (sugars), and lipids [8-11, 12]. Minerals with antioxidant effects include selenium, zinc, copper, sulfur, and iron. The use of DPPH free radicals, one of the typical methods to assess the free radical inhibition

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potential of antioxidant molecules, was first conducted by Belius in 1958. This method is widely used to measure the antioxidant activity of plants, fruits, flowers, oil seeds, and roots [8]. Prostate cancer is a common cancer in men, which mainly occurs in the peripheral area of the gland [13]. Symptoms of prostate cancer include frequent urination, blood in the urine, painful ejaculation, and impotence [14]. In this study, the antioxidant activity of the flower extract of the Cyprus Rotundus by the DPPH method, two solvents, ethanol and dimethyl sulfoxide (DMSO), and the number of minerals with antioxidant effects by the ICP-MS device, and the anticancer activity of flower extract on prostate cancer cells was evaluated.

MATERIAL AND METHODOS

Chemicals

In this study, the materials that utilized existed 2,2-diphenyl-1-picrylhydrazyl (DPPH) sourced from Sigma Aldrich (Germany), dimethyl sulfoxide (DMSO) as the solvent, and 90% ethanol. Ascorbic acid (vitamin C) was obtained from Merck (Germany), while MTT was procured from Carl Roth (Germany). Phosphate-buffered saline (PBS) was acquired from Sigma Aldrich (Germany), and fetal bovine serum (FBS) was provided by Gibco (USA). Trypsin-EDTA sourced from Bio-Idea (Iran). PC-3 cell line was obtained from the Pasteur Institute of Iran.

Plant Sample Preparation

In October, Cyperus Rotundus flowers were collected from Khuzestan Province, Iran. They were identified and confirmed by the Khuzestan Province Agriculture and Natural Resources Research and Education Center with herbarium code 11127. The flowers were dried in the shade and away from sunlight, and then they were micro-powdered with an electric grinder model 1048 and 05 or 500 MESH.

Plant Extract Preparation

To prepare the extract by maceration method, 25 grams of flower powder was blended in 250 ml of distilled water solvent and put on a shaker machine in an environment away from sunlight for 48 hours. The obtained extract was passed through filter paper using a Buchner funnel. The solvent was removed using an oven at a temperature of 50°C.

DPPH Test

To measure the antioxidant capacity of the extract and inhibit free radicals, the researchers utilized the DPPH free radical trapping method, and the IC50 value was calculated using graphs 2, 4, and 6. In this study, the final findings were analyzed using Graph Pad Prism 9 software and $p < 0.05$ was considered significant. For the ethanol extract by Serial Dilution method, concentrations of 1250, 625, 312.5, 156.25, 78.12, 39.06, 19.53, 9.76 and 4.88 µg/ml were prepared in ethanol. To prepare the second extract by Serial Dilution method, the concentrations of 1250, 625, 312.5, 156.25, 78.12, 39.06, 19.53, 9.76 and 4.88 µg/ml in dimethyl sulfoxide (DMSO) were prepared. Also, to prepare the initial stock of vitamin C in ethanol, concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml were prepared. To perform this test, 1 ml of DPPH solution was added to 2.5 ml of each dilution prepared from ethanol extract, dimethyl sulfoxide extract and vitamin C, and blank and control solutions were prepared along with them. Then the solutions were incubated for 30 minutes in a dark place at room temperature [15]. After this time, their absorption was measured using a spectrophotometer at 518 nm. The inhibition percentage of DPPH free radicals was calculated with the following formula:

% Inhibition of DPPH=(Ac-As)/Ac × 100

Measuring Minerals with Antioxidant Properties

The prepared extract was used to measure the amount of minerals with ICP-MS model ELAN6100DRC-e manufactured by Perkin Elmer company by inductively coupled plasma-mass spectrometry method [16].

MTT Test

PC-3 cell line was used for this test, and this cell line is isolated from human prostate cancer grade 4 epithelial tissue cells (adenocarcinoma grade 4), which have entered the stage of metastasis to bone tissues [17]. It was maintained in RPMI 1640 medium, 2 mM glutamine, and 10% Fetal Bovine Serum. Cell passage or subculture technique was used to grow and multiply the cell line [18]. The MTT method was used to evaluate the medicinal effect of the extract on prostate cancer cells. Cancer cells were treated with the mentioned concentrations for 24, 48 and 72 hours and the results were analyzed. First, the initial stock was prepared in phosphate buffer saline (as a solvent) with an amount of 3200 µg/ml, and then the concentrations of 25, 50, 100, 200, 400, 800, 1600 µg/ml were prepared from the initial stock of the extract by Serial Dilution method. In the first step, 200 µl of the cell suspension containing 7000 cells was poured into each well of the 96-well plate using a sampler (7 concentrations, 5 repetitions). In three prepared plates, 7 control wells and 7 blank wells were considered in each plate. In the second step, cancer cells were treated with the mentioned concentrations with a multi-channel sampler in each plate. In the third step, the three plates prepared by the above method were placed separately, the first for 24 hours, the second for 48 hours, and the third for 72 hours in an incubator at a temperature of 37°C, 5% CO_2 and 90% humidity. In the fourth step and after the mentioned period, three plates were removed from the incubator, the culture medium was emptied and MTT solution was added to each well to form formazan crystals and placed in the incubator for 4 hours. In the fifth step in the dark, DMSO solvent was added to each well to dissolve the formazan crystals and placed again in the incubator for 15 minutes. In the sixth step, all the wells were pipetted once and the absorbance was measured at 570 nm. Finally, using the following formula, the percentage of cell viability was calculated for the drug concentration [14]. The final findings were analyzed using Graph Pad Prism 9 software and $p < 0.05$ was considered significant.

% Cell viability=Average absorption of each treatment/Average absorbance of control × 100

RESULTS

Antioxidant Activity

According to Fig 1, the reduction of IC50 value from 935.8 µg/ml in ethanol solvent to 51.65 µg/ml in dimethyl sulfoxide solvent indicated that the antioxidant property of the extract was dependent on concentration and solvent. In Fig 2, the comparison of IC50 between the

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three groups showed that the combination of extract and ethanol solvent with vitamin C standard had a significant difference ($p<0.0001$****), the combination of extract and dimethyl sulfoxide solvent with vitamin C standard had a significant difference ($p<0.0001$****), the combination of extract and dimethyl sulfoxide solvent with the combination of extract and ethanol solvent had a significant difference ($p<0.0001$****). In Fig 3, the comparison between the percentage of inhibition of free radicals in similar concentrations of two groups, the combination of extract and dimethyl sulfoxide solvent with the combination of extract and ethanol solvent had a significant difference ($p<0.0001$****). The data and findings showed the effect of DMSO solvent on increasing the antioxidant property of plant extract compared to ethanol solvent. Also, in Table 1, the comparison between minerals from the highest to the lowest amount, respectively, of magnesium, sulfur, iron, manganese and zinc affected the antioxidant activity of the extract. (P-Value ($p<0.05$*, $p<0.01$**, $p<0.001$***, $p<0.0001$****))

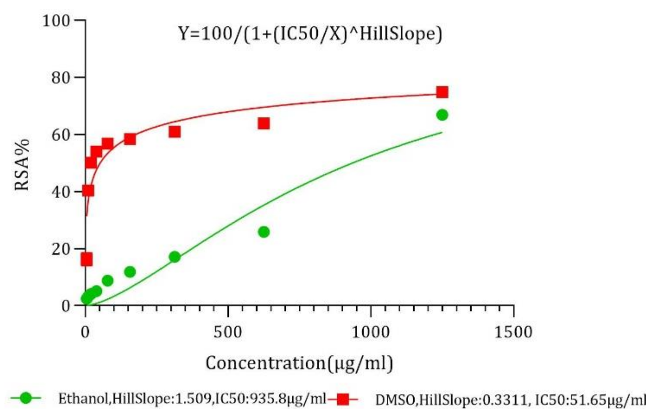

Fig. 1 Comparison of the antioxidant effect between the combination of extract with ethanol solvent and extract with dimethyl sulfoxide solvent according to IC50

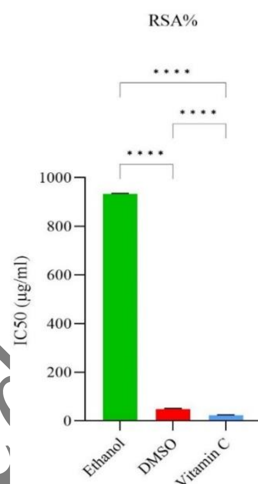

Fig. 2 Comparison of antioxidant effect between extract with ethanol solvent and extract with dimethyl sulfoxide solvent with vitamin C standard according to IC50

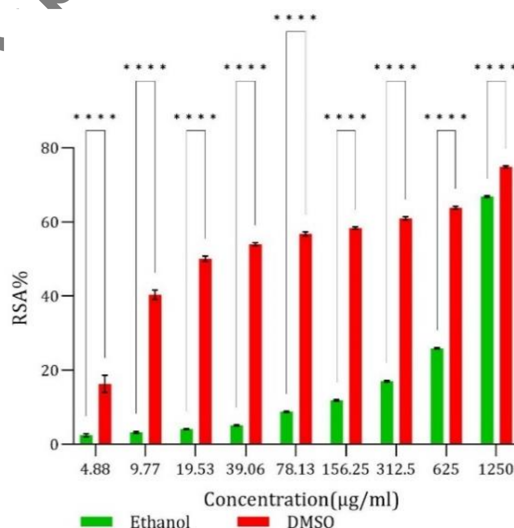

Fig. 3 Comparison between the percentage of inhibition of free radicals in similar concentrations between two groups, the combination of extract and dimethyl sulfoxide solvent with the combination of extract and ethanol solvent

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Table 1 Findings from the analysis of minerals with antioxidant properties by ICP-MS

Minerals with Antioxidant Properties	Amount (ppm)
Magnesium	16121.36
Sulfur	15885.44
Iron	499.22
Manganese	186.01
Zinc	135.43
Selenium	< 0.1
Copper	< 0.1

Anticancer Activity

According to Fig 4 and 5, the decrease of IC50 value from 904.5 μg/ml in 24 hours to 408.7 μg/ml in 48 hours and 252.7 μg/ml in 72 hours shows that the toxicity of the drug was dependent on the dose (concentration) and time, and IC50 at three times of 24, 48 and 72 hours has a significant difference ($p<0.0001$****). According to Fig 6, among the drug doses, the doses of 800 and 1600 μg/ml had a more lethal effect on cancer cells, and the cell viability of cancer cells in 24 hours of treatment was a significant difference between the control and the mentioned concentrations ($p<0.05$*) and at 48 and 72 hours of treatment, there was a significant difference between the control and the mentioned concentrations ($p<0.01$**). In Figure 7, the cell viability percentage of cancer cells in the same concentrations of two groups, 24 and 72 hours, showed that there is a significant difference between the concentrations of 800 μg/ml and the concentrations of 1600 μg/ml ($p<0.0001$****). (P-Value ($p<0.05$*, $p<0.01$**, $p<0.001$***, $p<0.0001$****))

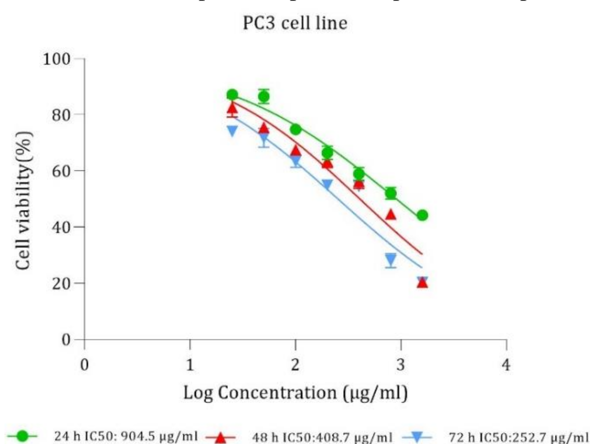

Fig. 4 Comparison between the cell viability percentage of cancer cells in three times of 24, 48 and 72 hours according to IC50

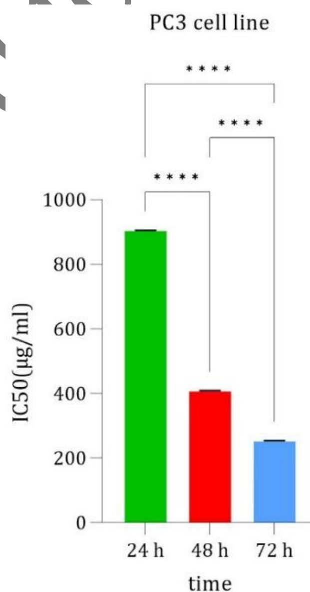

Fig. 5 Comparison of IC50 between three times of 24, 48 and 72 hours

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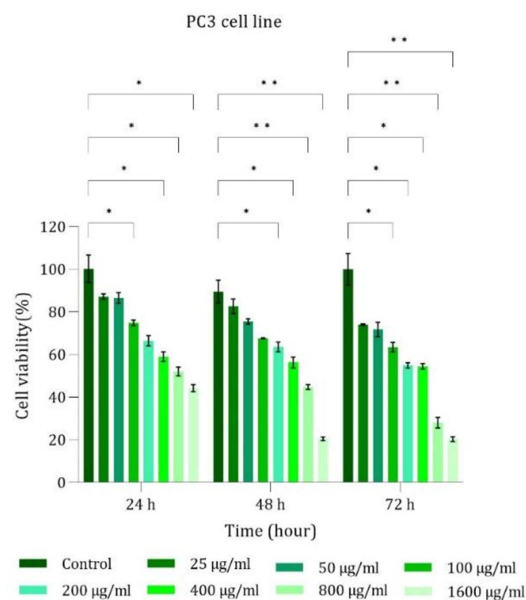

Fig. 6 Comparison of the cell viability percentage of cancer cells between control and different concentrations in each treatment (24 hours of treatment, 48 hours of treatment and 72 hours of treatment)

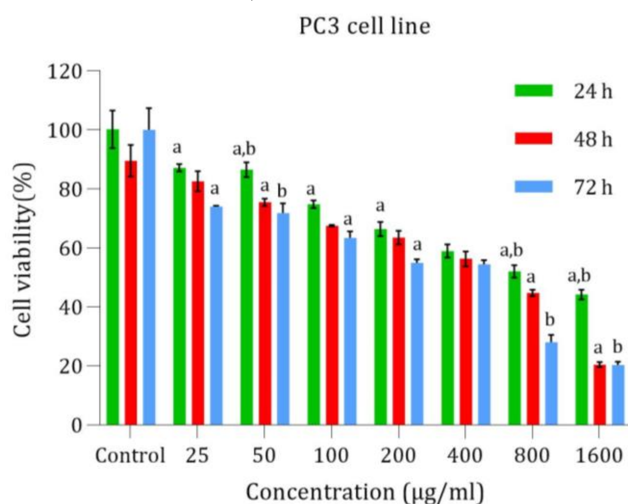

Fig. 7 Comparison of the cell viability percentage of cancer cells in similar concentrations between three times 24, 48 and 72 hours of treatment. Similar letters on the columns indicate statistically significant differences between groups.

DISCUSSION

This study was conducted to investigate the antioxidant properties of extracts derived from the tubers of the Cyperus rotundus plant, specifically focusing on their ability to inhibit superoxide free radicals ($O_2^{\bullet-}$) The findings indicated that the extract enriched with flavonoid oligomers, obtained through maceration method and infusion, demonstrated a superior capacity to inhibit 50% of free radicals at a lower concentration compared to other extracts, including those derived from ethyl acetate and methanol, as well as the control compound, quercetin. This was established based on the IC50 values calculated for each extract. The IC50 of different extracts and control quercetin has been reported as the extract enriched with flavonoid oligomers at a concentration of 68 µg/ml, the tail extract at a concentration of more than 1000 µg/ml, the ethyl acetate extract at a concentration of 90 µg/ml, the methanol extract at a higher concentration of 1000 µg/ml and control quercetin at a concentration of 360 µg/ml inhibited 50% of free radicals [19]. In a study, the antioxidant effects of Cyperus Rotundus plant tuber extracts were investigated and the findings were as follows among the methanol, ethyl acetate, chloroform, hexane and aqueous extracts compared to the ascorbic acid control, the methanolic extract at a lower concentration than other extracts (28.35 µg/ml), inhibited 50 % of free radicals. Between the methanolic extract and the ascorbic acid control, the control sample at a lower concentration, inhibited 50% of free radicals [20]. Dimethyl sulfoxide traps hydroxyl free radicals and its reducing metabolite dimethyl sulfide traps oxygen free radicals. It is believed that their ability to inhibit free radicals causes anti-inflammatory properties. Many studies show that dimethyl sulfoxide, in addition to being a solvent, has various biological effects such as antioxidant, anti-inflammatory and antiseptic properties [8]. So far, no research has been done on the flower extract of the Cyperus Rotundus plant in the field of antioxidant activity. This study investigates the antioxidant activity of Cyperus Rotundus flower extract using the DPPH method, employing two solvents: ethanol and dimethyl sulfoxide (DMSO). Additionally, the influence of DMSO on enhancing the antioxidant properties of the plant extract has been evaluated. Vitamin C was utilized as a control and standard reference in this analysis. Antioxidant content based on IC50, the aqueous extract of the Cyperus Rotundus flower with ethanol solvent is 935.8 µg/ml, with DMSO solvent 51.65 µg/ml and for vitamin C, 24.9 µg/ml. In comparing the IC50 of all three cases, vitamin C had more antioxidant properties and DMSO solvent had a

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great effect in increasing this property by reducing the IC50 value of the extract compared to ethanol solvent. In Fig 3, the increase in the antioxidant property of different concentrations of flower extract is evident, which indicates the synergy of the antioxidant property of dimethyl sulfoxide solvent and extract compared to ethanol and extract. According to the value of IC50, in the mentioned studies, the alcoholic extract of methanol in the amount of more than 1000 and the amount of 28.35 μg/ml, but in the present study, the alcoholic extract of ethanol in the amount of less than 1000 and more than 28.35 μg/ml, inhibited 50 % of free radicals. Selenium is the main antioxidant that combines with protein to form selenoprotein. Selenoprotein is an important antioxidant found in brains, seafood, red meat, and liver [21, 22]. Zinc is an essential catalytic, structural, and regulatory element that plays important roles in homeostasis, immune responses, oxidative stress, and aging. Zinc metal also does not directly affect free radicals, but is quite important in preventing their formation. Zinc causes the production of metallothionein, which is a hydroxyl radical inhibitor. The antioxidant role of copper is in its catalytic function in copper-dependent superoxide dismutase (Cu-SOD). Also, sulfur is considered an antioxidant due to its cooperation with enzymes that reduce organic peroxides. Catalase is the most abundant iron-containing antioxidant enzyme, which converts hydrogen peroxide into water and molecular oxygen in two steps. In the review of minerals with antioxidant properties, two elements manganese (Mn) and magnesium (Mg) have been mentioned [8]. Research indicates that the antioxidant properties of the Cyperus Rotundus flower extract can be enhanced by the presence of certain minerals. Specifically, measurements revealed that magnesium, sulfur, iron, manganese, and zinc contribute significantly to the antioxidant content of this extract. In a study on the toxic effect of the Cyperus Rotundus plant rhizome extract on prostate cancer cells (PC-3), the findings were reported as follows: petroleum ether, methylene chloride, ethyl acetate, butanol and water extracts, the most toxic effect based on the IC50 value respectively, methylene chloride, petroleum ether, and butanol extracts had concentrations less than 500 μg/ml, ethyl acetate extracts had concentrations less than 1000 μg/ml, and aqueous extracts had concentrations less than 2000 μg/ml [23]. In a study on the toxic effect of the essential oil of the rhizome of the Cyperus Rotundus plant on prostate cancer cells (PC-3) based on the IC50 value, 3444 ± 2.64 μg/ml had a toxic effect on the mentioned cell line [24]. So far, there has been no research on the toxic activity of the Cyperus Rotundus flower extract on prostate cancer cells. The comparison between the mentioned studies and the present study has shown that the aqueous extract of the plant flower had a more toxic effect on this cell line than the aqueous extract of the rhizome of the plant and essential oil.

CONCLUSION

In the investigation of antioxidant activity and cellular toxicity of plant flower extract, the findings showed that the antioxidant activity of flower extract in the presence of DMSO solvent was more than that of ethanol solvent, but vitamin C as a control sample had more antioxidant effect than both. Also, by adding dimethyl sulfoxide solvent to the flower extract, the antioxidant property has increased in all concentrations. The flower extract had a toxic effect on prostate cancer cells and caused a decrease in the cell viability of cancer cells and an increase in cell death. In the investigation of the amount of minerals to solve the lack of minerals in the body, it is believed that the flower extract contains the necessary amount of minerals according to the body's daily need for them, and more research is needed. Also, some minerals with antioxidant properties had a positive effect on increasing the antioxidant content of the plant.

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REFERENCES

1. Ghafari F., Naseri M., Khodadoost M. Iranian traditional medicine and the reasons for its revival and development. Medicine and Tezkieh Journal. 2010;3:63-71.
2. Ahmadi A. Pharmacological Probable effects of Cinnamon (Cinnamomum) on the treatment of people with Coronavirus 2019 (Covid 19). th [5] International Conference On Food Industry Sciences. 2020;2.
3. Mozaffarian V., Ramezani A. Identification of Iranian weeds, Shahrab and Ayandesazan. 2021.
4. Imam H., Sofi G., Aziz S., Lone A. The incredible benefits of Nagarmotha (Cyperus Rotundus). International journal of Nutrition Pharmacology Neurological Diseases. 2014;4(1):23-27.
5. Kumar M., Rani M., Meher B. Review on Pharmacology and Phytochemistry of Cyperus Rotundus L. Current Research in Pharmaceutical Sciences. 2017;7(1):11-15.
6. Mozaffarian V. Knowing the medicinal and aromatic plants of Iran, Farhang Moaser. 2017.
7. Madanipour M.M., Sharifi A. Investigation of chemical compounds and antioxidant activity of Cydonia Oblonga leaf extract. Journal of Innovation in Food Science and Technology. 2017;10(1):109-119.
8. Ahmadi A. Principles and basics of phytochemistry, Elm and Danesh. 2023.
9. Sayedi Z.S. Oxidative stress and human diseases. Iranian Journal of Biology. 2022;5(10):135-141.
10. Salmaninezhad A., Kangari P., Shakori A. Oxidative stress: progression and development of breast cancer: a review article. Journal of Faculty of Medicine. 2017;75(1):1-9.
11. Craft B.D., Kerrihard A.L., Amarowicz R., Pegg R.B. Phenol-Based Antioxidants and the In Vitro Methods Used for Their Assessment. Comprehensive Reviews in Food Science and Food Safety. 2012;11(2):148-173.
12. Carocho M., Ferreira I.C.F.R. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and Chemical Toxicology. 2013;51:15-25.
13. Hassanzadeh G., Soleimani Rad J., Fazlipour S., Nouri Moghi M.H., Golden I., Monsafi M., Orazhizadeh M., Ghorbani R., single planter N., Dehghani F., Abbasi M., Malik F., Majidi Zolbin M., Mokhtari T., Mehrayin F., Golalipour M.C. Basic histology of John Quira, Ibn Sina Publications. 2012.

14. Jalilian S. Investigating the effect of colchicine on apoptotic pathways in PC-3 prostate cancer cell line, Zabul University. 2015.
15. Faizi P. Isolation, purification and structure determination of compounds in Johreniopsis Stricticaulis plant and investigation of its antibacterial and antioxidant properties and cytotoxicity on MCF7 and PC-3 cancer cell lines, Payam Noor University. 2018.
16. Taheranjad M., Salahinejad M. Practical Inductively Coupled Plasma Spectroscopy (John R. Dean), Research Institute of Nuclear Sciences and Technologies. 2014.
17. Ahmadi A. Phytochemical investigation of Cyperus Rotundus L and investigation of cytotoxicity, antioxidant and antimicrobial effects of this plant, Yasouj University. 2023.
18. Izadi Fard F. Studying the cellular effects of the ethanolic extract of the aerial parts of the medicinal plant Barijeh (Ferula gummosa) on prostate cancer cells (PC-3), Mazandaran University. 2019.
19. Kilani S., Ben Sghaier M., Limem I., Bouhlel I., Boubaker J., Bhouri W., Skandrani I., Neffatti A., Ben Ammarb R., Dijoux Franca M.G., Ghedira K., Chekir Ghedira L. In vitro evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of Cyperus Rotundus. Bioresource Technology. 2008;99(18):9004-9008.
20. Mannarreddya P., Denis M., Munireddy D., Pandurangan R., Thangavelu K.P., Venkatesan K. Cytotoxic effect of Cyperus Rotundus rhizome extract on human cancer cell Lines. Journal Biomed Pharmacother. 2017;95:1375–1387.
21. Hoshmand S. Man, nutrition, health Volume II Minerals, Education and promotion of agriculture. 2014.
22. Nam Mian P. Investigation of antitumour, antimicrobial and antioxidant properties of Nerium Oleander. Mohaghegh Ardabili University. 2011.
23. Samra R.M., Soliman A.F., Zaki A.A., Ashour A., Al-Karmalawy A.A., Hassan M.A., Zaghloul A.M. Bioassay-guided isolation of a new cytotoxic ceramide from Cyperus Rotundus L. South African Journal of Botany. 2021;139:210-216.
24. Samra R.M., Soliman A.F., Zaki A.A., El-Gendy A.N., Hassan M.A., Zaghloul A.M. Chemical Composition, Antiviral and Cytotoxic Activities of Essential Oil from Cyperus Rotundus Growing in Egypt: Evidence from Chemometrics Analysis. Bearing Plants. 2020;23(4):648-659.

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