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Evaluation of Antioxidant Constituents and Cytotoxic Effects of *Rumex Tuberosus* Leaf Extract on Colorectal Cancer Cells

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Abstract

Colon cancer is one of the most common causes of mortality worldwide and has been identified as the second leading cause of cancer-related deaths among adults. Therefore, there is an urgent need to control this malignancy using therapeutic approaches with minimal side effects. The present study aimed to identify the antioxidant compounds in the leaf extract of (*Rumex Tuberosus*) and to evaluate its inhibitory effects on the growth of colorectal cancer cells. For this purpose, the leaves of *R. Tuberosus* were collected from Tonekabon, air-dried in the shade, and ground into powder. The extracts were prepared by maceration, and the total phenolic, flavonoid, and anthocyanin contents were determined. The HT29 colorectal cancer cell line was cultured and treated with various concentrations of the leaf extract (ranging from 62.5 to 2000 µg/ml) for 24, 48, and 72 hours. Cell viability and cytotoxicity were assessed using the MTT colorimetric assay. The results showed that the leaf extract of *R. Tuberosus* was rich in phenolic compounds, with the highest content of 17.19 ± 1.015 mg/g dry weight. Moreover, the MTT assay revealed a concentration- and time-dependent decrease in colorectal cancer cell viability, with the maximum inhibition (87.86%) observed at 2000 µg/ml after 72 hours of incubation. These findings suggest that the leaf extract of *Rumex Tuberosus* possesses notable anticancer properties and may serve as a promising candidate for further development in pharmaceutical applications.

Keywords: *Rumex Tuberosus*, Antioxidant compounds, MTT assay, Colorectal cancer, Total phenolic content.

1 | Introduction

Colon cancer is one of the leading causes of mortality worldwide and has been reported as the second most common cause of cancer-related death among adults. Current therapeutic approaches for the management and treatment of this disease include surgery and chemotherapy, both of which are often associated with severe side effects and can damage healthy cells. Consequently, researchers worldwide are seeking alternative

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methods that achieve maximum therapeutic efficacy with minimal adverse effects [1]. One promising approach involves using bioactive compounds derived from medicinal plants. In recent years, extensive efforts have been made to identify natural antioxidants from fruits and green leafy vegetables with strong potential to combat life-threatening diseases of the modern era [2]. Numerous studies have demonstrated that natural antioxidant compounds have a high capacity to scavenge and neutralize free radicals and reactive oxygen species. Therefore, plants rich in antioxidant compounds can serve as potential therapeutic agents in the treatment of diseases caused by oxidative stress [3], [4]. Among leafy plants, several species of the genus *Rumex*, commonly known as sorrels, are of particular interest. Although these plants are typically wild and not widely cultivated, they are traditionally collected from natural habitats during their short growing seasons and consumed locally as edible herbs. *Rumex Tuberosus*, commonly referred to as wild sorrel, is a herbaceous plant belonging to the family Polygonaceae. More than 250 species of the *Rumex* genus have been reported globally, with approximately 200 species distributed across temperate regions of the Northern and Southern Hemispheres. In Iran, over 23 species of *Rumex* have been identified, predominantly growing in the western regions, the Alborz and Zagros mountain ranges, and the Azerbaijan area. Different *Rumex* species are valued worldwide for their nutritional and medicinal properties.

In traditional medicine, the leaves and roots of *R. Tuberosus* have been used to treat constipation, hypertension, baldness, tumors, colds, inflammation, bleeding, and headaches. The plant is also recommended as a natural source of vitamin C supplementation. The pharmacological importance of the *Rumex* genus primarily arises from its biological activities, including antimicrobial, anti-inflammatory, antipyretic, antidiarrheal, and antiviral effects. Its roots also possess therapeutic properties and have been used to treat coughs, fevers, and headaches. Moreover, the extract exhibits astringent effects, has been applied in the treatment of dysentery, and chewing the fresh tuber is traditionally believed to relieve a sore throat. The roots are known to be laxative, tonic, anti-rheumatic, and beneficial for skin disorders [5]. Given the high nutritional and medicinal value of wild sorrel (*Rumex Tuberosus*), the present study aimed to evaluate the antioxidant properties of its leaf extract and investigate its potential anticancer activity against colorectal cancer cells.

2 | Materials and Methods

2.1 | Plant Collection and Extraction by Maceration

The leaves of *Rumex Tuberosus* (wild sorrel) were collected during the summer of 2023 from a region in Tonekabon County, Iran, at an altitude of 55 meters above sea level. The plant samples were air-dried in the shade and then ground into fine powder. Subsequently, 50 g of the powdered sample was soaked in 400 mL of 80% methanol and kept at room temperature for 24 hours. After the extraction period, the mixtures were filtered, and the solvent was evaporated under reduced pressure at temperatures below 40°C using a rotary evaporator. The obtained extracts were stored in a refrigerator at 4°C until further analysis [6].

2.2 | Determination of Total Phenolic, Flavonoid, and Anthocyanin Content

2.2.1 | Total Phenolic Content

The total phenolic content of the extracts was determined using the Folin Ciocalteu colorimetric method [7]. Briefly, 100 µL of the plant extract was mixed with 2 mL of 2% sodium carbonate solution, 2.8 mL of distilled water, and 100 µL of 50% Folin Ciocalteu reagent. After 30 minutes of incubation at room temperature, the absorbance of the reaction mixture was measured at 720 nm against a blank. Gallic acid was used as the standard, and the total phenolic content was expressed as milligrams of Gallic Acid Equivalents (mg GAE) per gram of dry plant weight.

2.2.2 | Total Flavonoid content

The total flavonoid content was measured according to the aluminum chloride colorimetric method described by Chang et al. [8]. In this procedure, 500 µL of each extract was mixed with 1.5 mL of 80% methanol, 100 µL of 1 M potassium acetate, 100 µL of 10% aluminum chloride solution, and 2.8 mL of distilled water. After

40 minutes of incubation at room temperature, the absorbance was recorded at 415 nm using a spectrophotometer. Quercetin was used to construct the calibration curve, and results were expressed as milligrams of Quercetin Equivalents (mg QE) per gram of dry plant weight.

2.2.3|Total Anthocyanin content

The total anthocyanin content was determined following the method of Mita et al. [9]. 0.05 grams of dried plant tissue were homogenized with 4 mL of 1% HCl in methanol using a porcelain mortar. The resulting mixture was stored at 4°C in a refrigerator for 24 hours, then centrifuged at 13,000 rpm for 10 minutes. The absorbance of the supernatant was measured at 530 and 657 nm against a blank of 1% HCl in methanol. The anthocyanin content of each extract was calculated using *Eq. (1)*:

$$\text{Total Anthocyanin content } A = A_{530} - (0.25 \times A_{657}),$$

A: solution absorbance (the subscript numbers indicate the wavelengths at which absorbance readings were taken.)

2.3|Investigation of the Effects of Wild Sorrel (*Rumex Tuberosus*) Leaf Extract on the Growth and Viability of Colorectal Cancer Cells

The HT29 colorectal cancer cell line was obtained from the Pasteur Institute of Iran. To evaluate the effects of the methanolic leaf extract of *Rumex Tuberosus* on cell growth and viability, the MTT colorimetric assay (5,4,3-dimethylthiazol-2-yl-5,2-diphenyltetrazolium) was employed [10]. Briefly, 10^4 cells were seeded in each well of 96-well plates. After 24 hours of incubation, the cells were treated with various concentrations of the extract (62.5, 125, 250, 500, 1000, and 2000 µg/mL) for 24, 48, and 72 hours. At each time point, the absorbance of the wells was measured at 540 nm using an ELISA plate reader. Cell viability was calculated according to the following formula [10]:

$$\text{Cell Viability (\%)} = \text{Absorbance of Sample} / \text{Absorbance of Control} \times 100.$$

2.4|Statistical Analysis

All data are presented as mean $\pm$ standard error of three replicates. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Duncan's multiple range test in SPSS software. Differences were considered statistically significant at $p < 0.05$. Graphs were plotted using Microsoft Excel 2010.

3|Results

3.1|Total Phenolic, Flavonoid, and Anthocyanin Contents of Wild Sorrel Leaf Extract

The results indicated that the *Rumex Tuberosus* leaf extract was rich in antioxidant compounds, particularly phenolics, with the highest phenolic content observed in the leaf extract. The total phenolic content was measured at 17.19 ± 1.015 mg Gallic Acid Equivalents (GAE) per gram of dry plant weight. In comparison, the total flavonoid and anthocyanin contents in the extract were relatively low *Table 1*.

Table 1. Total phenolic, flavonoid, and anthocyanin contents of wild sorrel (*Rumex Tuberosus*) leaf extract. Data are presented as mean $\pm$ standard error.

Total Anthocyanins (mg g ⁻¹ DW)	Total Flavonoids (mg EQ g ⁻¹ DW)	Total Phenolics (mg EGA g ⁻¹ DW)	Plant Part
0.045 $\pm$ 0.001	0.155 $\pm$ 0.019	17.19 $\pm$ 1.015	Leaf

3.2 | Evaluation of the Effects of Different Concentrations of Wild Sorrel (*Rumex Tuberosus*) Leaf Extract on Colorectal Cancer Cell Viability Over 24 Hours

Overall, the effects of different concentrations of *Rumex Tuberosus* leaf extract on colorectal cancer cell viability were significant compared with the control. A significant reduction in cell viability was observed at extract concentrations of 500 $\mu\text{g/mL}$ and above. The lowest cell viability ($26.98 \pm 1.411\%$) was recorded at the highest concentration tested (2000 $\mu\text{g/mL}$), indicating substantial inhibition of cancer cell growth. Additionally, the IC_{50} of the leaf extract for HT29 cells after 24 hours was determined to be 224 $\mu\text{g/mL}$ (Fig. 1).

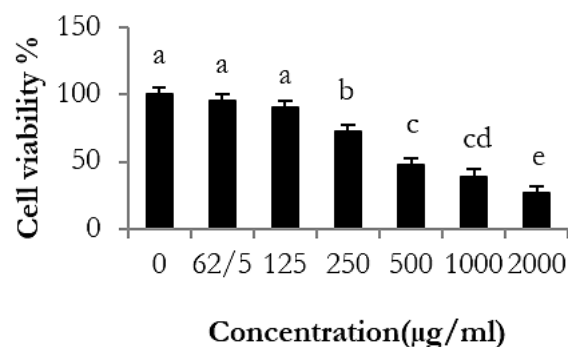


Fig. 1. Effect of different concentrations of wild sorrel (*Rumex Tuberosus*) leaf extract on colorectal cancer cell viability over 24 hours.

In Fig. 1, Each bar represents the mean $\pm$ standard deviation. Bars sharing at least one letter, according to Duncan's multiple range test at the 5% probability level, are not significantly different.

3.3 | Effects of Different Concentrations of Wild Sorrel (*Rumex Tuberosus*) Leaf Extract on Colorectal Cancer Cell Viability over 48 Hours

The results indicated that varying concentrations of *Rumex Tuberosus* leaf extract significantly affected the viability of colorectal cancer cells compared to the control. A significant reduction in cell viability was observed at extract concentrations of 250 $\mu\text{g/mL}$ and above. The lowest cell viability ($15.34 \pm 0.981\%$) was recorded at the highest tested concentration (2000 $\mu\text{g/mL}$), demonstrating potent inhibition of cancer cell growth. The IC_{50} value of the leaf extract for HT29 cells after 48 hours was determined to be 623.5 $\mu\text{g/mL}$ (Fig. 2).

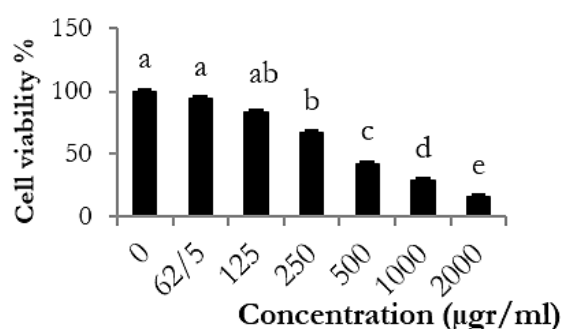


Fig. 2. Comparison of the effects of different concentrations of wild sorrel (*Rumex Tuberosus*) leaf extract on colorectal cancer cell viability over 24 hours.

In Fig. 2, Each bar represents the mean $\pm$ standard deviation. Bars sharing at least one letter, according to Duncan's multiple range test at the 5% probability level, are not significantly different.

3.4 | Evaluation of the Effects of Different Concentrations of Wild Sorrel (*Rumex Tuberosus*) Leaf Extract on Colorectal Cancer Cell Viability Over 72 Hours

This study demonstrated that different concentrations of wild sorrel (*Rumex Tuberosus*) leaf extract significantly inhibited the growth of colorectal cancer cells. A notable reduction in cell viability was observed at an extract concentration of 125 $\mu\text{g}/\text{mL}$ compared to the control. The lowest cell viability ($12.14 \pm 0.751\%$) was recorded at the highest tested concentration (2000 $\mu\text{g}/\text{mL}$). This reduction was more pronounced at 72 hours than at 24 hours, indicating a time-dependent inhibitory effect. Furthermore, the IC_{50} value of the leaf extract for HT29 cells after 72 hours was determined to be 415.4 $\mu\text{g}/\text{mL}$ Fig. 3.

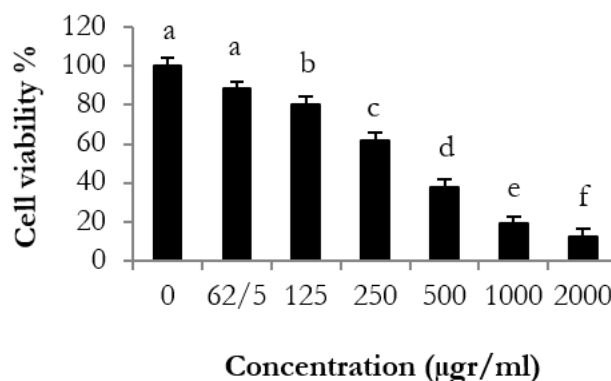


Fig. 3. Comparison of the effects of different concentrations of wild sorrel (*Rumex Tuberosus*) leaf extract on colorectal cancer cell viability over 72 hours.

In Fig. 3, Each bar represents the mean $\pm$ standard deviation. Bars sharing at least one letter, according to Duncan's multiple range test at the 5% probability level, are not significantly different.

4 | Discussion

Colon cancer is recognized as one of the most common causes of mortality in adults. Given the inability of conventional chemotherapeutic drugs to fully treat this disease, which has resulted in numerous deaths, there is a pressing need to identify alternative strategies to inhibit malignancy. This has prompted researchers to investigate medicinal plants for their potential to reduce or inhibit cancer cell growth. Extensive studies aimed at identifying bioactive compounds in medicinal plants have demonstrated that consuming these plants can reduce tumor formation and carcinogenesis in the colon. The present study showed that the effect of *Rumex Tuberosus* leaf extract on colorectal cancer cell viability was both concentration- and time-dependent (Figs. 1-3). Different concentrations of the leaf extract reduced cancer cell viability, with the most significant decrease observed at 2000 $\mu\text{g}/\text{mL}$ after 72 hours. The results also indicated that the leaf extract is rich in antioxidant compounds, particularly phenolic constituents. These compounds may inhibit the cell cycle or activate its checkpoints, prevent DNA replication, and trigger intrinsic or extrinsic apoptotic pathways. Furthermore, they can induce differentiation and cell death in leukemia and lung cancer cells, suppress tumor angiogenesis, and inhibit metastasis or cell proliferation [11]. The ester groups present in phenolic compounds can reduce oxidative stress and protect against cellular damage.

The antioxidant properties of phenolic compounds protect cells against oxidative damage and, through their antiproliferative effects, can prevent the transformation of normal cells into cancerous cells [12]. Arast et al. [13] reported that antioxidants can stimulate immune cells to localize at tumor sites, eliminate cancer cells, or inhibit angiogenesis. Previous studies have shown that antioxidant compounds, such as phenolic acids, polyphenols, and flavonoids, scavenge free radicals, including hydroperoxides, superoxide, and hydrogen peroxide, thereby preventing oxidative processes that lead to genomic damage and mutations. Flavonoids, in

particular, interfere with immune and cellular processes associated with cancer progression, such as cell proliferation, differentiation, and angiogenesis [3], [14].

In a related study, the anticancer effects of *Rumex confertus* extract on cell viability and molecular pathways in MCF-7 breast cancer cells were investigated. The study demonstrated that the hexane extract significantly reduced cell viability, with an IC_{50} of 9.40 $\mu\text{g/mL}$ after 96 hours. Molecular analyses indicated upregulation of genes involved in apoptosis. The findings also suggested that wild sorrel extract may induce a less differentiated, stem-like state in cancer cells, potentially enhancing resistance to malignancy [15].

Based on the studies reviewed, it can be concluded that antioxidant compounds can reduce proliferation and increase apoptosis in cancer cells. In the present study, the mechanism of action of *Rumex tuberosus* leaf extract in inhibiting colorectal cancer cells was not investigated; therefore, no definitive conclusions can be drawn. However, this study confirmed that the leaf extract is rich in antioxidant compounds, particularly phenolics. Given the significant positive correlation between phenolic content and inhibition of cancer cell growth, it is likely that these phenolic compounds, as antioxidants, may contribute to the inhibition of cancer cell proliferation, potentially through activation of programmed cell death pathways.

5 | Conclusion

The results of this study demonstrated that wild sorrel *Rumex Tuberosus* leaves are rich in phenolic compounds. Furthermore, the leaf extract exhibited concentration- and time-dependent inhibitory effects on colorectal cancer cell growth, with the highest inhibition observed at 6222 $\mu\text{g/mL}$ after 48 hours. Based on these findings, it can be concluded that wild sorrel leaf extracts are abundant in antioxidant compounds. Given the side effects associated with conventional chemotherapeutic drugs, the leaf extract of this plant is recommended for further pharmacological investigation and has potential for future therapeutic applications.

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