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Antioxidant and Anticancer Activities of the Flower Extract of Rhynchocorys Elephas on A549 Lung Cancer Cells

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Abstract

Lung cancer is one of the most common types of cancer, and chemotherapy remains a main therapeutic approach; however, its severe side effects often limit its use. Therefore, the present study aimed to determine the antioxidant compounds of Rhynchocorys Elephas flower extract and to evaluate its inhibitory effects on the growth of A549 lung cancer cells. The flowers of R. Elephas were collected from Tonekabon, air-dried in the shade, and powdered. The extracts were prepared by maceration, and the total phenolic, flavonoid, and anthocyanin contents were quantified. The A549 cell line was cultured and exposed to various concentrations of the flower extract (62.5–2000 μ g/mL) for 24, 48, and 72 hours. Cytotoxicity was then assessed using the MTT colorimetric assay. The results showed that the R. Elephas flower extract was rich in antioxidant compounds, particularly phenolics, with the highest concentration (13.98 ± 0.269 mg/g dry weight) observed in the extract. Moreover, MTT assay results revealed that cell viability decreased in a concentration- and time-dependent manner. The highest cytotoxic effect was observed at 2000 μ g/mL after 72 hours of incubation, where cell viability decreased to 80.12%. Based on these findings, the flower extract of R. Elephas is a rich source of antioxidant compounds and, considering the adverse effects of chemotherapy drugs, can be recommended for further pharmacological investigations as a potential natural anticancer agent.

Keywords: Antioxidant compounds, MTT assay, Lung cancer, Rhynchocorys Elephas, Total phenolics.

1 | Introduction

Antioxidants play a crucial role in inhibiting free radicals and breaking the oxidative chain reactions. The inhibition of oxidation processes in food, pharmaceutical, cosmetic, and hygienic products, as well as the prevention of diseases related to oxidative stress, are among the beneficial functions of antioxidants [1].

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However, the use of synthetic antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) has been reported to cause undesirable side effects, including carcinogenicity [2].

Currently, cancer is the second leading cause of death after cardiovascular diseases. Lung cancer is the most prevalent type of cancer worldwide and is considered an epidemic. In 2002, more than 1.3 million people were diagnosed with lung cancer, accounting for approximately 29% of all cancer-related deaths [3]. Various therapeutic approaches are used in cancer treatment, including surgery, chemotherapy, radiotherapy, immunotherapy, and others. Systemic chemotherapy remains one of the primary treatments for lung cancer; however, the lack of selective toxicity often leads to intolerable side effects.

In addition to conventional methods, the use of medicinal plants in cancer therapy has gained considerable attention [4]. Accordingly, extensive research has been conducted on the potential use of plant-derived antimicrobial and anticancer compounds to control and treat pathogenic diseases [5], [6]. Due to the prevalence of drug resistance and the reduced efficacy of synthetic compounds, plant-based natural products have attracted significant interest, leading major pharmaceutical companies worldwide to invest heavily in this area. Medicinal plants, containing both enzymatic and non-enzymatic antioxidant compounds, can neutralize and inhibit free radicals and Reactive Oxygen Species (ROS) [7]. Damage caused by ROS contributes to the development of diseases such as cancers and cardiovascular disorders. The beneficial effects of antioxidant compounds on human health are mainly attributed to their ability to reduce oxidative stress [8]. Studies have shown that certain antioxidant compounds, such as phenolics and flavonoids, possess antimicrobial, anti-apoptotic, and anti-proliferative properties [9].

Rhynchocorys Elephas, commonly known as the "elephant-flower", belongs to the Scrophulariaceae family. It produces attractive yellow flowers shaped like an elephant's trunk, which gives it its local name. In Gilan Province (northern Iran), the plant blooms in early spring (March–April) and grows abundantly in meadows, along roadsides, and in lowland areas. The chemical constituents of R. Elephas include saponins, flavonoids, and tannins, and the plant is traditionally known for its antimicrobial and anti-inflammatory properties. The flower extract of R. Elephas has been reported to exhibit antifungal, antibacterial, and antioxidant activities [10].

Given the rapid expansion of R. Elephas in northern Iran and the limited studies on its pharmacological properties, the present study aimed to investigate the antioxidant profile of R. Elephas flower extract and to evaluate its cytotoxic effects on lung cancer cells.

2 | Materials and Methods

2.1 | Plant Collection and Extraction by Maceration Method

The flowers of Rhynchocorys Elephas were collected from a region in Tonekabon County (northern Iran) during the summer of 2024 at an altitude of approximately 50 meters above sea level. The samples were air-dried in the shade and then ground into a fine powder. For extraction, 50 g of the powdered sample was soaked in 200 mL of 80% methanol and kept at room temperature for 48 hours. After the extraction period, the mixture was filtered, and the solvent was evaporated at a temperature below 40°C using a rotary evaporator. The resulting crude extract was stored at 4°C in a refrigerator until further analysis [11].

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

2.2.1 | Total Phenolic content

To determine the Total Phenolic Content (TPC), 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 incubation for 30 minutes, the absorbance was measured at 720 nm against a blank. Gallic acid was used as

the standard for constructing the calibration curve, and the TPC was expressed as milligrams of Gallic Acid Equivalent (GAE) per gram of dry weight (mg GAE/g DW) [12].

2.2.2 | Total Flavonoid content

For the determination of total flavonoids, 500 µL of each extract was mixed with 1.5 mL of 80% methanol, 100 µL of 10% aluminum chloride, 100 µL of 1 M potassium acetate, and 2.8 mL of distilled water. After 40 minutes of incubation, the absorbance was measured at 415 nm against a blank. Quercetin was used as the standard, and the Total Flavonoid Content (TFC) was expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW) [13].

2.2.3 | Total Anthocyanin content

To measure Total Anthocyanin Content (TAC), 0.05 g of dried plant material was ground in a mortar with 4 mL of 1% HCl in methanol. The mixture was refrigerated for 24 hours, then centrifuged at 13,000 rpm for 10 minutes. The absorbance of the supernatant was recorded at 530 and 657 nm against a blank containing 1% HCl in methanol. The anthocyanin content for each extract was calculated using *Eq. (1)* [14]:

$$A = A_{530} - (0.25 \times A_{657}),$$

where A is the absorbance value, and the subscripts represent the respective wavelengths used for measurement.

2.3 | Evaluation of the Cytotoxic Effect of Rhynchocorys Elephas Leaf Extract on A549 Lung Cancer Cells

The human lung carcinoma cell line A549 was obtained from the Pasteur Institute of Iran. The cytotoxic and growth inhibitory effects of the methanolic extract of Rhynchocorys Elephas leaves were evaluated using the MTT colorimetric assay (5,4,3-dimethylthiazol-2-yl-5,2-diphenyltetrazolium). For this purpose, 1×10^4 cells were seeded into each well of a 96-well microplate. After 24 hours of incubation to allow cell attachment, different concentrations of the extract (62.5, 125, 250, 500, 1000, and 2000 µg/mL) were added to the wells for incubation periods of 24, 48, and 72 hours. At the end of each incubation period, cell viability was assessed by measuring absorbance at 540 nm using an ELISA microplate reader. The percentage of cell viability was calculated according to *Eq. (2)* [15]:

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

2.4 | Statistical Analysis

All data were expressed as the mean ± Standard Error (SE) of three independent replicates. Statistical analyses were performed using Analysis of Variance (ANOVA), and mean comparisons were conducted using Duncan's multiple range test at a significance level of $p < 0.05$. Statistical analyses were conducted using SPSS, and graphs were prepared in Microsoft Excel 2010.

3 | Results

3.1 | Total Phenolic, Flavonoid, and Anthocyanin Contents of Rhynchocorys Elephas Flower Extract

The results indicated that the Rhynchocorys Elephas flower extract was rich in antioxidant compounds, particularly phenolic compounds. The TPC of the flower extract was 13.98 ± 0.269 mg GAE per gram of dry weight. In comparison, the total flavonoid and anthocyanin contents in the leaf extract of R. Elephas were relatively low (See *Table 1*).

Table 1. Total phenolic, flavonoid, and anthocyanin contents of Rhynchocorys Elephas extracts.

Total Anthocyanins (mg g^{-1} DW)	Total Flavonoids (mg EQ g^{-1} DW)	Total Phenolics (mg EGA g^{-1} DW)	Plant Part
0.026±0.001	0.205±0.024	13.98 ± 0.269	Leaf

Data are presented as mean ± SE.

3.2 | Effect of Different Concentrations of Rhynchocorys Elephas Flower Extract on A549 Lung Cancer Cell Viability at 24 Hours

The results of this study demonstrated that increasing the concentration of the extract decreased the viability of A549 lung cancer cells compared to the control. This reduction became statistically significant at concentrations of 500 µg/mL and above. The greatest decrease in cell viability was observed at 2000 µg/mL, where cell viability was 46.84 ± 4.40%, corresponding to 53.16% inhibition. The IC_{50} value for A549 cells at this time point was calculated as 1723.7 µg/mL (See *Fig. 1*).

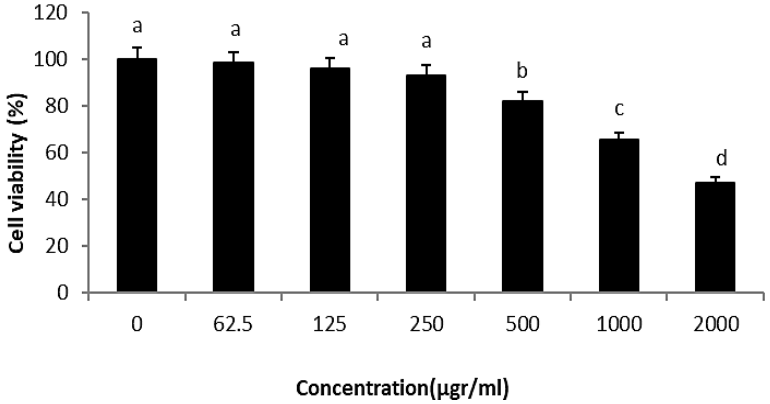

Fig. 1. Effect of different concentrations of Rhynchocorys Elephas flower extract on the viability of A549 lung cancer cells after 24 hours.

In *Fig. 1*, Each bar represents the mean ± Standard Deviation (SD). Bars sharing at least one letter are not significantly different according to Duncan's multiple range test at $p < 0.05$.

3.3 | Effect of Different Concentrations of Rhynchocorys Elephas Flower Extract on A549 Lung Cancer Cell Viability at 48 Hours

The results indicated that various concentrations of Rhynchocorys Elephas flower extract, particularly 500–2000 µg/mL, had a significant inhibitory effect on A549 lung cancer cell growth compared to the control. The lowest cell viability was observed at 2000 µg/mL, reaching 35.78 ± 4.07%, corresponding to 64.22% inhibition. At 1000 µg/mL, the extract also caused a significant reduction in cancer cell growth compared with the control. The IC_{50} value for A549 cells at 48 hours was calculated as 1522.4 µg/mL (See *Fig. 2*).

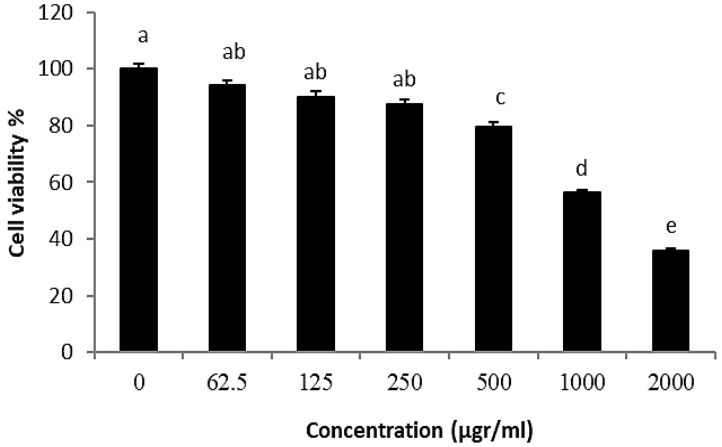

Fig. 2. Effect of different concentrations of Rhynchocorys Elephas flower extract on the viability of A549 lung cancer cells after 48 hours.

In *Fig. 2*, Each bar represents the mean ± SD. Bars sharing at least one letter are not significantly different according to Duncan's multiple range test at $p < 0.05$.

3.4 | Effect of Different Concentrations of Rhynchocorys Elephas Flower Extract on A549 Lung Cancer Cell Viability at 72 Hours

The results demonstrated that various concentrations of Rhynchocorys Elephas flower extract significantly inhibited the growth of A549 lung cancer cells. Increasing the extract concentration from 250 to 2000 µg/mL resulted in a marked decrease in cell viability compared to the control. The most significant reduction in cell viability was observed at 2000 µg/mL, reaching 19.88 ± 1.14%, corresponding to 80.12% inhibition, which was notably higher than the inhibition observed at 24 hours. Additionally, concentrations of 500 and 1000 µg/mL also caused a considerable reduction in cancer cell viability relative to the control. The IC_{50} value for A549 cells at 72 hours was calculated as 1236.2 µg/mL (See *Fig. 3*).

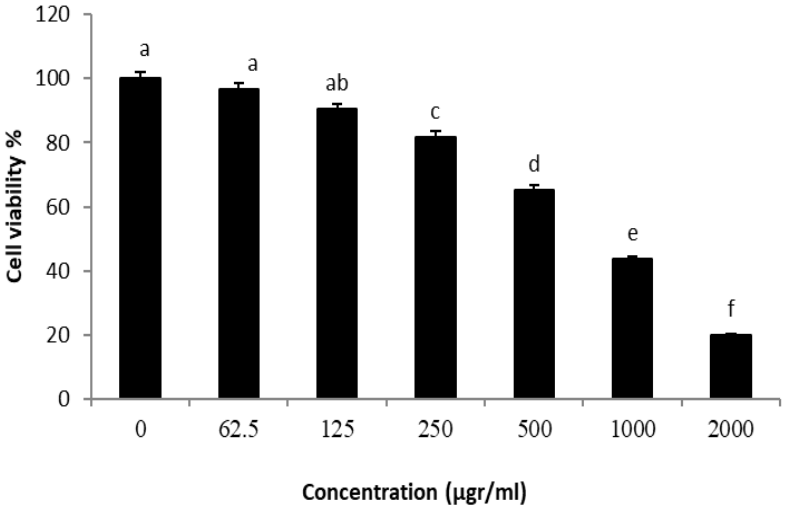

Fig. 3. Effect of different concentrations of Rhynchocorys Elephas flower extract on the viability of A549 lung cancer cells after 72 hours.

In *Fig. 3*, Each bar represents the mean ± SD. Bars sharing at least one letter are not significantly different according to Duncan's multiple range test at $p < 0.05$.

4 | Discussion

In recent years, many drugs used to treat various malignancies have been of plant origin. The use of natural plant-derived compounds for cancer management has attracted considerable attention due to their low side effects and promising therapeutic potential. Numerous studies have investigated the anticancer effects of medicinal and endemic plants in different countries [7], [16], [17].

The present study demonstrated that Rhynchocorys Elephas flower extract affected the viability of A549 lung cancer cells in a concentration- and time-dependent manner (See *Figs. 1–3*). Different concentrations of the flower extract reduced cancer cell viability, with the most significant reduction observed at 2000 µg/mL after 72 hours. The results also indicated that the flower extract contains high amounts of antioxidant compounds, particularly phenolic constituents. These compounds may inhibit the cell cycle or activate checkpoints, interfere with DNA replication, or trigger intrinsic and extrinsic apoptotic pathways. Additionally, they may induce differentiation and cell death in leukemia and lung cancer cells, suppress tumor angiogenesis, and reduce metastasis or cellular migration [18].

Previous studies have shown that antioxidant compounds, such as phenolic acids, polyphenols, and flavonoids, scavenge free radicals, including hydroperoxide, superoxide, and hydrogen peroxide, thereby preventing oxidative processes that lead to genomic damage and mutations. Other reports indicate that polyhydroxylated flavonoids, such as quercetin, inhibit cancer cell growth in vitro by reducing DNA synthesis by approximately 14% compared to control groups and by arresting cells in the G1 phase of the cell cycle [7].

These findings are consistent with the present study and support a significant positive correlation between the anticancer activity of extracts and their phenolic content. Based on these collective studies, it can be concluded that phenolic compounds may reduce proliferation and induce apoptosis in lung cancer cells. Although the precise genetic pathways underlying the anticancer mechanism of R. Elephas flower extract were not investigated in this study, our results demonstrate that the extract is rich in antioxidants, particularly phenolic compounds, which likely contribute to the inhibition of lung cancer cell growth via programmed cell death pathways.

5 | Conclusion

The results of this study demonstrated that the flower extract of Rhynchocorys Elephas is rich in phenolic compounds. Furthermore, the extract exhibited a concentration- and time-dependent inhibitory effect on the growth of A549 lung cancer cells, with the highest inhibition observed at 2000 µg/mL after 72 hours. Therefore, given the high antioxidant content of the flower extract and the side effects associated with conventional chemotherapy, R. Elephas flower extract is recommended for further pharmacological investigations and has potential for future applications in cancer therapy.

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