Bioactive Composition, Antioxidant, And Cytotoxic Activities of *Rheum Ribes* Extracts

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**A R T I C L E  I N F O**

**ABSTRACT**

The aim of the study is to evaluate the ethanol extract of *Rheum ribes* root (RTE), as well as the root and young shoots (RYSE), for its chemical composition, antioxidant properties and cytotoxic effects. Total polyphenols (TPC), total flavonoid (TFC), radical scavenging activity (DPPH), the ferric reducing antioxidant power (FRAP) of *Rheum ribes* extracts were determined using colorimetric methods. Mineral contents and vitamin values of samples were determined by ICP-MS and HPLC, respectively. The cytotoxic effect of *Rheum ribes* extracts was determined on different cells using XTT assay. The cytotoxic effects of RTE and RYSE on cancer cells were evaluated with regard to apoptosis. According to results, mineral contents, vitamine A and C values were very high in both samples. Ethanolic extracts of *Rheum ribes* young shoot exhibited a selective cytotoxic effect on all cancer cells compared to WI-38 cells, and the IC50 values of the extract in the cancer cells between 26.10 to 54.81 µg/mL. Ethanolic extracts of RTE and RYSE induced apoptosis on MCF-7 cells. The ethanolic extracts of *Rheum ribes* has effective cytotoxic and antioxidant activity. More research is needed to determine the cytotoxic effect mechanisms on cancer cells.

**Keywords:** Antioxidant activity, Cytotoxicity, HPLC, ICP-MS, Rheum ribes

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**Introduction**

Natural products have been utilized to heal a variety of disorders since ancient times. It is known as alternative or ethnic medicine, the medicines used are mostly derived from natural products (Atanasov et al., 2021; Yuan et al., 2016). In recent years, the emergence of the side effects of drugs has led people to consume natural products known as drugs (Tan et al., 2011). Natural products are considered as potential sources for the discovery of new drugs and active ingredients of drugs (Atanasov et al., 2021; Tan et al., 2011).

*Rheum* species from the Polygonaceae family are also widely used in pharmacological research (Kashiyada et al., 1988). *Rheum ribes* (*R. ribes*) has flavonoids, anthrones, alkyl glucosides, stilbenes, emodin, rhein, aloe-emodin, crysophanol, and other compounds. *R. ribes* has many biological activities, such as antioxidative, antibacterial, and anticancer (AI-Shammari et al., 2020; Tartik et al., 2015). The biological effects of *Rheum* species are due to their high bioactive content (Kashiyada et al., 1988; Shokravi et al., 1997; Baytop, 1999; Abu-Irmaileh et al., 2003; Tabata et al., 1994; Bati et al., 2020). Its fresh stems and leaves are consumed as raw and cooked in Lebanon, Iran and eastern Turkey (Krishnaiah et al., 2011). In traditional treatment, while *Rheum ribes* is used as a diuretic in Turkey, it has been reported to be used as a sedative in Iran (Sayyah et al., 2009). Moreover, it is known to be used for hypertension, obesity, smallpox, cholagouge, measles, hemorrhoids, and stomach problems (Baytop, 1999; Abu-Irmaileh et al., 2003). In the literature, it has been shown that *R. ribes* root, stem, leaf, seed and flower have important biological activities in different solvent fractions (Sedigheh et al., 2005; Alan et al., 2012). Pyrocatechol and quer cetin have been shown to be important phenolic components in the ethanol extract of Rheum roots (Oztürk et al., 2007). Le et al. stated that emodin compound, which is one of the active components of Rheum species, induces apoptosis in human lung squamous carcinoma (Lee, 2001). There are limited studies on the cytotoxic and anticancer activity of root and young shoot extracts of *R. ribes* (Uyar et al., 2014).

In the present study, we investigate the biological activities of ethanol extract of *Rheum ribes* root (RTE), and root and young shoots (RYSE) such as antioxidant activity, and cytotoxic effects in various cancer cells. In addition, chemical composition of RTE, and RYSE extracts were determined.
Materials and Methods

**Extract Preparation**

*Rheum ribes* was collected from Iğdır in Turkey. The roots, and root and young shoots of *Rheum ribes* were dried in an oven at 42°C. Approximately 100 g of dried root, and root young shoot samples were extracted with 1000 mL ethanol in a mechanical shaker at 25°C for 72 h. After incubation, samples passed through 0.2 µm filters, and evaporated to dryness in vacuo to remove ethanol. The prepared RTE and RYSE were stored in a dry condition.

**Total Phenolic Contents (TPC)**

The Folin Ciocalteu method was used to determine the total polyphenol content of *Rheum ribes* (Sląk-Kuziel et al., 1977). In a basic solution, phosphotungstic acid (H3P[W3O10]4) is reduced to phosphotungstic blue to create the method. Gallic acid equivalents (GAE) were used to express the results in mg(GAE)/g of sample.

**Total Flavonoid Contents (TFC)**

The aluminum chloride colorimetric method was used to calculate the TFC of the *Rheum ribes* extract (Chang et al., 2002). In this method 80% ethanol, 10% Al (NO3)3 solution, and 1 M KCH3COO solution were added by adding the sample. Quercetin is used as the standard for expressing the total flavonoid content, and values were expressed as mg quercetin equivalents (QE)/g sample.

**Ferric Reducing Antioxidant Power assay (FRAP)**

The FRAP of the extract was determined by Oyaizu et al. method. The color of the test solution in this technique changes from yellow to green depending on the reducing power of the tested sample. Trolox served as the standard. The results were expressed as mg trolox equivalents (TE)/g powder (Oyaizu, 1986).

**Radical Scavenging Activity (DPPH)**

By the color of the test solution in this technique changes from yellow to green depending on the reducing power of the sample modifying the method developed by Ou et al. (Ou 2002) the radical scavenging activity of *Rheum ribes* extracts was determined. When treated by antioxidant substance or substances, the strength of the purple color arising from DPPH is reduced. Ascorbic acid was used as the standard. The results are given in μg/mL, after calculating the IC50 value.

**HPLC Analysis of Vitamin Compounds**

HPLC consisted of a Varian 9010 pump and a Varian 9050 UV detector. Twenty microliters were injected into C18 (Bonda Pack C18 30 cm x 3.9 mm). The mobile phase was acetonitrile: methanol: dichloromethane (7:0, 5:3). There is a 1.3 mL/min flow. The wavelength for vitamin A was set to 310 nm. Ammonium acetate pH 5.0-3 mM; acetonitrile (90%) served as the mobile phase. 1 mL per minute flow rate. The wavelength for vitamin C and B groups was set to 250 nm (www.sielc.com).

**Determination of mineral contents**

**Sample preparation**

The One Touch Technique was used to perform triplicate digestions on each plant sample. This approach involved adding 10 and 2 mL of HNO3 and HCl, respectively, to 0.5 g of duplicate samples that were transferred to an Xpress vessel (CEM) for microwave digestion. After acid mixture was added to vessels, the samples were allowed to pre-digest for 15 min without sealing process. The digestion of samples was carried out by following in one stage with power ranging from 1030-1800 W, ramp time of 20 to 25 min, hold time of 15 min and temperature of 200°C. After digestion procedure was completed, caps of vessels were opened carefully. Then, volume of the digested samples was completed to 50 mL by ultrapure water, and stored in refrigeration until the analysis.

**ICP-MS analysis**

The concentrations of various elements in plant extracts were analyzed by ICP-MS (Inductively Coupled Plasma-Mass Spectrometer). The digested samples prepared by microwave extraction were submitted to ICP system for analysis.

All samples were subjected to sp-ICP-MS analysis to ascertain the particle size and concentration using an ICP-Q ICP-MS (Thermo-Scientific Waltham, MA, USA) outfitted with standard nickel sampling and skimmer cones, standard glass concentric nebulizer, quartz spray chamber, and small internal diameter (1.0 mm) quartz torch. Samples were put directly into the ICP-MS using a peristaltic pump and tubing as per usual procedure (i.d. 1.02 mm). There was no settling time between measurements and analyses were carried out in time-resolved analysis (TRA) mode with an integration period (dwell time) of 100 s per point. The summary of the instrumental parameters utilized for the ICP-MS analysis is provided below. RF power (1550 W), Carrier gas (0.67 L/min) Spray chamber temperature (2 °C), Nebulizer pump (0.1 rps), Sample depth (8.0 mm), Integration time (100 μs), Acquisition time (60 s).

**Cell culture**

Human prostate cancer (DU-145, HTB-81), rat glioma cell (C6, CCL-107), human colorectal adenocarcinoma (Caco2, HTB-37), human breast cancer cell (MCF-7, HTB22), human hepatocellular carcinoma (HepG2, HB-8065), human bone osteosarcoma (MG63, CRL-1427), and normal human lung cell (WI-38, CCL-75) cells were purchased from America Type Culture Collection (ATCC). All cells were cultured in DMEM, containing 10% fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin-streptomycin with 5% CO2 at 37°C in an incubator.

**XTT assay**

The cytotoxic effect of RTE and RYSE on different cells was determined by XTT assay 24 h after treatment. After incubation, absorbance was measured at 475 nm (Hepokur et al., 2019). All values were compared to control samples (untreated group) which represented 100% viability.

**Apoptosis analysis**

The apoptotic effects of RTE and RYSE on MCF-7 and WI-38 cells were performed using a commercial kit (Muse® Annexin V & Dead Cell Kit, MCH100105, Germany). The MCF-7 and WI-38 cells were treated with IC50 values of ethanolic extracts of *Rheum ribes* for 24 h. The cells were washed with phosphate buffer saline (PBS), treated with trypsin-EDTA, and centrifuged at 800 rpm for 10 min. The binding buffer was then used to resuspension the cells. Treated cells were examined by flow cytometry after incubation.
Statistical Analysis

SPSS version 22.0 was used to analyze the experimental data (SPSS Inc., Chicago, USA). The results of each experiment were carried out in triplicate, and they were presented as mean standard deviation. One-Way ANOVA was performed to analyze intergroup differences. Significances were considered as those with a p<0.05 significant values.

Results and Discussion

Since a long history, Rheum species have been used as medicinal plants in traditional medicine. Rheum species has an important biological activity, such as antitumor, antioxidant, blood sugar lowering, antibacterial antifungal, laxative, diuretic, wound healing (Al-Shammari et al., 2020; Tartik et al., 2015; Tyler et al., 1988). Many natural products have been shown to include antioxidative components, and these molecules have been utilized in vitro as reducing agents, active oxygen scavengers, or free radical inhibitors (Shagoon et al., 2016).

Various in vitro methods have been used to determine the antioxidant activities of natural product extracts (Jin et al., 2010). The radical scavengers TPC, TFC, FRAP, and DPPH were utilized in this investigation. The literature review revealed that few studies on Rheum species have been studied for their antioxidant activity. Ceylan et al. (2019) reported that TPC and TFC, FRAP values of methanol extracts of Rheum ribes was found 112.82 ± 11.68 (mg GAE/g), 2.50 ± 0.31 (mgQE/g), 42.50 ± 2.44 (µmol Fe/g), respectively. Ozturk et al. (2007) showed that TPC was 35.71, 25.91 µg PEs/mg extract, and TFC was 13.66, 16.23 µg QEs/mg extract in the stems and roots of Rheum ribes, respectively. Pyrocatechol and quercetin have been shown to be important phenolic components in the methanol extract of Rheum roots. In another study, TPC and TFC of aqueous extracts of Rheum ribes were found 82.24±1.54 µg GAE/mg extract, 6.56±0.68 µgQE/mg extract (Turan et al., 2013). Our study indicated that TPC, TFC, and FRAP values of RTE were found 18.64±0.25 mg GAE/g sample, 1.427 mg QE/g sample, and 15.64±4 mg TE/g sample, respectively. TPC, TFC, and FRAP values of RTE were found 24.057 mg GAE/g sample, 1.746 mg QE/g sample, and 12.657 mg TE/g sample, respectively. The TPC and TFC, FRAP values are shown in Table 1. Antioxidant activity results were small different from the literature. The differences may arise from the harvested region have, type of extraction method, solvent, and harvest season conditions (Turan et al., 2015).

Determination of free radical scavenging activity of natural bioactive compounds is used DPPH. Keser et al. (2019) showed that the water, and methanol extracts of Rheum ribes scavenging activity ratio of 93.49%, 95.86%, respectively. In another study, radical scavenging activity of Rheum ribes shoot and root ethyl acetate extracts IC50 values were found 206.28 µg/mL, and 10.92 µg/mL (Uyar et al., 2014). Our results showed that the IC50 values of RYSE and RTE were found 7.92, 14.78 µg/mL, respectively. These data reported that radical scavenging activity of Rheum ribes values was consistent with the literature.

Medicinal plants include important proteins, essential fatty acids, other biological molecules, which used in industrial and pharmaceutical applications. The essential secondary metabolites are vital for the growth and development of all cells (Uyar et al., 2014). Among the main active compounds of Rheum ribes are especially emodin, rhein, chrysophanol, alizarin, and citreorosein. Rheum ribes contains vitamin C, phenolic and flavonoid components, tannins, and anthraquinones (Shagoon et al., 2016). Raafat et al. (2021) showed that the aqueous extract of Rheum ribes contains rutin (31.5%), quercetin-3-Galactoside (21.4%), quercetin (9.1%), fisetin (3.9%), emodin (9.8%), chrysophanol (8.6%). In another study showed that the main components of flower extract Rheum ribes L. hexane extract were p-Cymene, germacrene-d, terpinolene, linoleic acid, and oleic acid (Shafaghat et al., 2015).

Vitamins are essential nutrients that are necessary for a variety of biochemical functions and which must be taken with the diet, because they can not be synthesized in the body. Especially green plants are the main sources of vitamins (Taşkın et al., 2019). According to this results we showed that Rheum ribes ethanol extracts includes vitamin C, A at significantly amounts. Vitamin A was found 2.005, 0.1059 ppm for RTE, and RYSE, respectively (Table 2). Vitamin C was found 49.2484 ppm for RTE, and 51.9152 ppm for RYSE (Table 2). Munzuroğlu et al. (2000) determined that the levels of A, E, and C vitamins of Rheum ribes L. were in the ranges of 0.255–0.363, 0.614–0.765, 197.6–282.3 µg·gr⁻¹, respectively. Mineral contents of Rheum ribes ethanol extracts were determined by ICP-MS. Chemical analysis results of the Rheum ribes samples were established to give nutrient values per ppm (Esctilli et al., 2014). The concentrations of aluminum, potassium, magnesium, calcium, sodium, and zinc are shown in Table 2.

Table 1. Results for ethanol extracts of Rheum ribes, including TPC, TFC, and FRAP values.

<table>
<thead>
<tr>
<th>Extract</th>
<th>RYSE</th>
<th>RTE</th>
</tr>
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<tbody>
<tr>
<td>TPC (mg Quercetin/g sample)</td>
<td>1.427±0.025</td>
<td>1.746±0.034</td>
</tr>
<tr>
<td>TFC (mg gallik asit/g sample)</td>
<td>18.644±0.427</td>
<td>24.057±2.074</td>
</tr>
<tr>
<td>FRAP (mg trolos/g sample)</td>
<td>15.644±0.96</td>
<td>12.657±0.25</td>
</tr>
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Table 2. Mineral contents and vitamins for ethanol extracts of Rheum ribes.

<table>
<thead>
<tr>
<th></th>
<th>Na</th>
<th>Mg</th>
<th>Al</th>
<th>Zn</th>
<th>K</th>
<th>Ca</th>
<th>A vit</th>
<th>C vit</th>
</tr>
</thead>
<tbody>
<tr>
<td>RYSE (ppm)</td>
<td>38.55</td>
<td>78.49</td>
<td>13.27</td>
<td>26.01</td>
<td>462.89</td>
<td>23.47</td>
<td>0.1059</td>
<td>51.9152</td>
</tr>
<tr>
<td>RTE (ppm)</td>
<td>37.41</td>
<td>66.50</td>
<td>12.75</td>
<td>25.90</td>
<td>420.01</td>
<td>55.89</td>
<td>0.2005</td>
<td>49.2484</td>
</tr>
</tbody>
</table>
Table 3. IC⁵₀ values of ethanolic extracts of *Rheum ribes* on different cell lines (n=3)

<table>
<thead>
<tr>
<th></th>
<th>HepG2</th>
<th>C6</th>
<th>MCF-7</th>
<th>MG63</th>
<th>Caco2</th>
<th>DU-145</th>
<th>WI-38</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC⁵₀</td>
<td>RYSE 32.99±1.02</td>
<td>38.80±0.65</td>
<td>21.72±0.68</td>
<td>32.99±1.15</td>
<td>29.00±0.24</td>
<td>54.81±1.46</td>
<td>65.42±1.65</td>
</tr>
<tr>
<td>(µg/mL)</td>
<td>RTE 52.95±0.62</td>
<td>33.58±1.69</td>
<td>30.43±1.95</td>
<td>30.14±0.65</td>
<td>49.47±2.45</td>
<td>74.83±2.48</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Annexin V-FITC analysis of RYSE and RTE-treated WI-38 and MCF-7 cells (n=3).

<table>
<thead>
<tr>
<th></th>
<th>WI-38</th>
<th>MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-apoptotic (%)</td>
<td>Early apoptotic (%)</td>
</tr>
<tr>
<td>RYSE</td>
<td>99.22±0.2</td>
<td>0.44±0.1</td>
</tr>
<tr>
<td>RTE</td>
<td>97.88±1.4</td>
<td>1.93±0.2</td>
</tr>
<tr>
<td>RYSE</td>
<td>25.31±1.4</td>
<td>69.42±0.1</td>
</tr>
<tr>
<td>RTE</td>
<td>27.06±0.1</td>
<td>67.29±0.1</td>
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Ozcan et al. (2009) showed that aluminum, calcium, iron, potassium, magnesium, sodium, phosphorus, and zinc values were very high in *Rheum ribes*. In this study, we determined that ethanol extracts of *Rheum ribes* were very rich in potassium and magnesium. Differences in mineral elements and vitamin content may be due to growing conditions, climate and analytical procedures.

Cancer is the most common cause of mortality worldwide and a serious health issue in the world. All most cancer chemotherapy compounds in clinical use have obtained from plants or are analogs agents. Natural products are important, especially in the drug discovery process. Recently, many researchers have focused on anticancer agents from traditional medicinal plants (Sharifi et al., 2019). In the literature, there are many studies showing that the cytotoxic effect of Rheum species ethyl acetate, aqueous, ethanolic, and methanolic extracts (Tarkan et al., 2015; Li et al., 2009; Nho et al., 2015; Esmaeilbeig et
al., 2015; Sardari et al., 2009). According to According to Tartik et al. (2015), the ethanol extract of *Rheum ribes* roots reduced PC3 cells ability to viability via having a pro-oxidant effect. Another study demonstrated that *Rheum officinale Bail* (Da Huang) has anticancer activity against MCF-7 and A549 cells, IC50 values found 620±12.7 and 515±10.1 μg/mL, respectively (Li et al., 2009). Sardari et al. (2009). showed that the IC50 values of *Rheum ribes* ethanol extract were found 25.37 mg/mL. AGS (human Caucasian gastric adenocarcinoma), 51.34 mg/mL MCF-7, 11.21 mg/mL, SKLC6 (human lung carcinoma), 46.41 mg/mL SW742 (human colorectal adenocarcinoma). Esmaeilbeig et al. (2015) investigated the cytotoxic activities of the *Rheum ribes* methanol extracts on different cancer cells. The study reported that the *Rheum ribes* methanol extract had notable effects on myelogenous leukemia (K562) cells. The IC50 value was found 115 μg/mL for K562 cells.

According to Nho et al. (2015), *Rheum palmatum L.* ethanol extract decreased MDA-MB-231 cells’ viability in a concentration-dependent manner. The cytotoxic effects of RTE and RYSE were determined on HepG2, C6, MCF-7, MDA-MB-231, Caco2, DU-145, and WI-38 cell lines using the XTT assay. Their values IC50 are shown in Table 3. Our results showed that RYSE has more cytotoxic effect on cell lines than RTE. The most selective cytotoxic effect of RYSE was found 3 in MCF-7 cells. RYSE and RTE exhibited promising anticancer activity against MCF-7 cells compared to another cells. Cytotoxic activity studies have shown similar results in the literature. It is accepted that all phenolic compounds, vitamins, and minerals of the *Rheum ribes* extracts contribute to its cytotoxic effect.

Apoptosis is important in both pathological and physiological processes and has a vital role in cancer treatment (Khodavirdipour et al., 2021). MCF-7 cells were used, in which RYSE and RTE showed the highest selective cytotoxic effect, to determine the effects of RYSE and RTE on apoptosis. Apoptosis analysis of RYSE and RTE were performed by flow cytometry. Our results demonstrated that IC50 concentration of RYSE and RTE induced cell apoptosis (Table 4, Figure 1). Lee et al. showed that the emodin component from Rheum species induces apoptosis in human lung squamous carcinoma (Lee, 2001). Furthermore, another compound, Aloe- emodin, induced apoptosis in colon cancer (Lin et al., 2010).

**Conclusion**

The obtained results in the present study shown that anethanolic extract of *Rheum ribes* has effective cytotoxic and radical scavenger activities. Further studies are needed to determine the mechanisms underlying cytotoxicity on cancer cells.

**Acknowledgment**

The plant extract in this study was obtained from World Medicine Drug Company A.S.

**Conflicts of interest**

None

**References**


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