MAUNSagBil.Derg. 2025;5(3) 1-12
https://dergipark.org.tr/tr/pub/maunsbd

Araştırma Makalesi/ Research Article

Elektroporasyonun Akciğer Kanseri Hücrelerinde Rheum Ribes ve Hypericum Perforatum Ekstratlarının Antiproliferatif Aktivitesi Üzerine Etkisi

Effect of Electroporation on Antiproliferative Activity of Rheum Ribes and Hypericum Perforatum Extracts in Lung Cancer Cells

Mehmet Eşref ALKIŞ[1], Fuat YETİŞSİN[2]

Özet: Bu çalışmanın amacı, Hypericum perforatum ve Rheum ribes bitkilerinin fenolik madde içeriklerini ve akciğer kanseri hücreleri (A549) üzerindeki sitotoksisitelerini belirlemek ve elektroporasyonun (EP) bu bitkilerin antiproliferatif aktiviteleri üzerindeki etkilerini ortaya koymaktır. Mevcut çalışma Hypericum perforatum ve Rheum ribes bitki özütlerinin akciğer kanseri hücreleri üzerinde elektroporasyonun etkisini araştıran ilk çalışmalardandır. Bitki ekstraktlarının antiproliferatif aktiviteleri A549 hücrelerinde, biyouyumlulukları ise L-929 fibroblast hücrelerinde MTT analizi ile test edilmiştir. A549 kanser hücrelerinin elektrokemoterapi (ekstraktlar+EP) uygulamalarında, 800V/cm yoğunluğunda sekiz kare dalga elektriksel puls dizisi, bitki ekstraktlarının çeşitli dozları ile birlikte kullanılmıştır. Her iki bitki ekstraktının da fenolik bileşikler bakımından zengin olduğu ve L-929 fibroblast hücreleri için 1000µg/mL'den daha yüksek IC50 değerleri ile neredeyse hiç sitotoksik etki göstermediği bulunmuştur. Bununla birlikte, A549 kanser hücreleri Rheum ribes ve Hypericum perforatum ekstraktlarının sitotoksik aktivitelerine karşı çok iyi duyarlılık göstermiş ve IC50 değerleri sırasıyla 297,32 ve 241,10 µg/mL olarak bulunmuştur. Her iki bitki ekstraktının sitotoksik aktivitesi EP ile önemli ölçüde artmış ve ekstrakt+EP gruplarının hücre canlılık yüzdelerinin sadece ekstrakt gruplarına kıyasla önemli ölçüde azaldığı görülmüştür ($p<0.05$). Bulgularımız, Rheum ribes ve Hypericum perforatum ekstraktlarının antikanser potansiyeline sahip olduğunu ve EP ile birlikte kullanıldığında akciğer kanseri için umut verici olabileceğini ortaya koymaktadır.

Anahtar Kelimeler: Akciğer Kanseri, Elektroporasyon, Fenolik Bileşikler, Sitotoksisite.

Abstract: The purpose of this study is to investigate the phenolic substance contents of Hypericum perforatum and Rheum ribes plants and their cytotoxicity on lung cancer cells (A549) and to reveal the effects of electroporation (EP) on the antiproliferative activities of these plants. The present study is one of the first to investigate the effect of electroporation of Hypericum perforatum and Rheum ribes plant extracts on lung cancer cells. Antiproliferative activities of plant extracts were determined in A549 cells, and their biocompatibility was evaluated in L-929 fibroblast cells through MTT assay. In electrochemotherapy (extracts+EP) applications of A549 cancer cells, eight square wave electrical pulse sequences with an intensity of 800V/cm were used with various doses of plant extracts. Both plant extracts were observed to be abundant in phenolic compounds and exhibited almost no cytotoxic effects, with IC50 values higher than 1000µg/mL for L-929 fibroblast cells. However, A549 cancer cells showed very good sensitivity to the cytotoxic activities of Rheum ribes and Hypericum perforatum extracts, with IC50 values of 297.32 and 241.10 µg/mL, respectively. The cytotoxic activity of both plant extracts increased considerably with EP, and cell viability percentages of extract+EP groups were observed to decrease significantly compared to extract-only groups ($p<0.05$). Our findings reveal that Rheum ribes and Hypericum perforatum extracts have anticancer potential and may be promising for lung cancer if used in combination with EP.

Keywords: Lung Cancer, Electroporation, Phenolic Compounds, Cytotoxicity.

[1] Muş Alparslan University, Department of Occupational Health and Safety, Faculty of Health Sciences Muş/Türkiye
[2] Muş Alparslan University, Department of Vegetable and Animal Production, Technical Sciences Vocational School Muş/Türkiye

Sorumlu yazar/ Corresponding Author: Mehmet Eşref ALKIŞ
Orcid ID: https://orcid.org/ 0000-0002-3321-2873
Adres/Address: Muş Alparslan University, Occupational Health and Safety, Faculty of Health Sciences
E-posta/e-mail: me.alkis@alparslan.edu.tr

Geliş tarihi/Received date: 06.02.2025
Düzeltme tarihi/Revision date: 15.05.2025
Kabul tarihi/Accepted date: 21.07.2025

Atıf: ALKIŞ M. E., YETİŞSİN F. Effect of Electroporation on Antiproliferative Activity of Rheum Ribes and Hypericum Perforatum Extracts in Lung Cancer Cells. MAUNSagBil.Derg.: 2025; 5(3); 1-12. DOI: 10.71255/maunsbd.1634531

INTRODUCTION

Lung cancer is one of the world's most frequent cancers and represents the primary cause of death related to cancer (Smolarz et al., 2025). Although treatment for this cancer usually includes targeted therapy, surgery, radiotherapy and chemotherapy, most of cases are detected at an advanced stage and have a dismal prognosis, with a five-year survival rate of around 15% (Bade and Dela, 2020). This cancer's incidence is quickly growing in several countries and is highly resistant to standard chemotherapy (Thapa et al., 2023). Most of the medications currently utilized in the lung cancer treatment do not have the desired effect and also have many side effects. Furthermore, despite developments in cancer treatment, diseases prognosis remains poor, emphasizing the requirement for innovative therapeutics for lung cancer patients (Islam et al., 2025). Novel therapies for lung cancer are expected to have few side effects, should protect healthy tissue as much as possible and shouldn't generate resistance to therapy in cancerous cells (Bendix et al., 2022). For these reasons, we need new chemotherapeutic agents and treatment methods to treat lung cancer.

The use of wild plants is recommended by many national and international organizations (WHO, UNEP, USDA etc.) due to their superior nutritional content and therapeutic properties. This situation prompts researchers to investigate the characteristics of conventional and edible wild plants (Alaca et al., 2022; Majitol et al., 2024). It has been observed that the researched plants have positive effects on health as a result of their consumption in the diet due to the secondary metabolites they contain. Hence, it is crucial to research the anticancer characteristics of these plants for use in lung cancer treatment. Although the anticancer effects of Rheum ribes and Hypericum perforatum extracts were previously shown in some cancer cells (Nehme et al., 2024), this issue has still not been fully elucidated.

Hypericum perforatum L., a perennial plant used for therapeutic purposes by the Greeks and Romans, is one of the most significant conventional and pharmaceutical agents produced in fields and collected from natural areas (Crockett and Robson, 2011; Kwiecień et al., 2021). In addition to the flavonoids, biflavonoids, xanthones, phenylpropanes, phenolics and polyphenols they produce in their secondary metabolism, they also synthesize hypericins and hyperforins specific to the hypricum genus. Anti-cancer, anti-oxidative, anti-microbial, anti-inflammatory and anti-depressant properties have been proven thanks to the abundance of secondary metabolite compositions (Bruni and Sacchetti, 2009; Napoli et al., 2018; Shakya et al., 2019). Additionally, alcoholic extracts of Hypericum perforatum have been recorded to exhibit antioxidant and anti-proliferative properties (Mirmalek et al., 2016; Chan et al., 2023). Rheum ribes L., a member of the Polygonaceae family, is rich in anthracene-derived compounds and, like Hypericum perforatum, has an important place in folk medicine (Erdoğan et al., 2020). It is known to have medicinal relevance in the treatment of numerous illnesses and ailments

such as diabetes, hypertension, obesity, ulcers, diarrhea, colds, hemorrhoids and flu (Öztürk et al., 2007; Hasani-Ranjbar et al., 2010; Akkol et al., 2011). It is also used as an anticancer plant, due to the secondary metabolites and vitamins B2, B1, A, C, K and E it contains (Munzuroğlu et al., 2000). In addition, Rheum ribes contains abundant secondary metabolites that may have anti-microbial and anticancer properties, such as flavonoids, anthocyanins, stilbene and anthraquinones (Li et al., 2008; Venkatadri et al., 2011; Magda and Nehad, 2011).

The efficiency of chemotherapy medications relies on their ability to penetrate the tumor cell effectively. Electrochemotherapy (ECT) offers a novel approach to combat the issue of cancer cells developing resistance to various medications (Condello et al., 2022). This treatment technique merges the delivery of chemotherapy with the use of short-term electric pulses (electroporation) and increases the uptake of drugs (Alkis et al., 2023). The safety, effectiveness, and feasibility of ECT have also been demonstrated in deeply embedded tumors such as lung tumors (Tremble et al., 2019) and can be well tolerated with little adverse effects (Probst et al., 2018). Moreover, the administration of local electric impulses to the tumor resulting in transient vasoconstriction and reduced blood flow within the tumor, thus is enhances the drug's effects by retaining the drug within the tumor for an extended period of time (Strojan et al., 2021). However, no studies have been found on how electroporation (EP) affects the cytotoxicity of Rheum ribes and Hypericum perforatum extracts in lung cancer.

Our aim in this investigation is to assess the phenolic substance contents of Hypericum perforatum and Rheum ribes plants and to examine their cytotoxic effects on A549 lung cancer cells and L-929 healthy cells using Chemotherapy and ECT treatment techniques.

MATERIALS AND METHODS

Plant Materials

Fresh plant of *Hypericum perforatum* was picked up from Muş Alparslan University campus (Muş) and *Rheum ribes* was collected from rural area near the Muş City (Eastern Anatolia region, Türkiye) in April and May 2023. The plants used in our study were identified according to Davis (Davis, 1985) Flora of Turkey. After the collected plant samples were taken to the laboratory, they were packaged in the freezer section of the refrigerator set at -20 °C to be used in the experimental stages.

Preparation of Extracts

After taking one g of the plant samples from the freezer, they were transferred to 50 mL falcon tubes and 10 mL of extraction solution (consisting of methanol, ethanol and pure water prepared in 1:1:1 ratio) was added. Then, the plant samples were completely fell aparted with a tissue lyser at 15 000 rpm (IKA, T18 digital Ultra Turrax, Germany). After the obtained extract was centrifuged at 500 rpm for 25 minutes at +4 ℃ degrees, the supernatant was transferred to 2 mL eppendorf tubes. The

extract was stored in the freezer at -20 ℃ degrees to be used in later cell culture studies (Azmir et al., 2013).

Measurement of Phenolics Content Composition

To assess the quantity of phenolic compounds using HPLC, the final concentrations of apigenin, abscisic acid, gallic acid, trans-p-coumaric acid, ascorbic acid, kaempferol, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, quercetin, myricetin acid, catechol, rosmarinic acid, cinnamic acid, caffeic acid and vanillin standards were measured with the goal to prepare solutions with a 10 milligrams per milliliter concentration. Next, the standards were supplemented with a 1% mixture of acetic acid and acetonitrile (at the rate of 9/1), and an equal amount of methanol was included in order to create the stock standards. The calibration curve was established using diluted stock standards at 10, 25, 50, 75, and 100 µg/mL concentrations (Seal, 2016). The plant leaf extracts' concentration was set to 20 mg per milliliter using the same solutions as in the standard. The extracts samples were filtered (0.45 micrometer membrane filter) and then placed into a HPLC machine. Agilent Technologies 1260 Infinity II HPLC machine was utilized for the HPLC analysis (Agilent, USA). The HPLC machine had a 1260 DAD WR detector with wavelengths of 272 nm, 280 nm, and 310 nm. It also had a 1260 Quat Pump VL pump with a flow rate of 1. 0 mL per minute, a 1260 Vial sampler that injected 20 µL, and a G7130A column furnace set at 28°C. Analysis was conducted using the ACE 5 C18 column (4.6x250 mm).

Antiproliferative Activity

Cell Culture and Reagents

A549 human lung cancer cell line and L-929 fibroblast cell line were employed in this investigation. The cells utilized in the research were acquired from Mus Alparslan University's Application and Research Center. Cell lines were cultured in DMEM media (Capricorn) mixed with pen-strept (100 IU/mL, 10 mg/mL) and 10% fetal bovine serum (FBS, Sigma) at 37 0C in a humidified environment with 5% CO2. Once the culture had grown to 85–90% confluence, removed from flask bottom with 0. 25% trypsin, 5 minutes centrifugated at 1300 rpm and used in experiments.

Cytotoxicity of Extracts

Ten thousand cells were seeded into each well of 96-well plates and placed in a carbon dioxide incubator for 24 hours. Following the incubation time, the DMEM inside the wells was removed and different doses of Hypericum perforatum and Rheum ribes extracts (0, 5, 25, 50, 100, 200 and 400 µg/mL) and doxorubicin (1, 5, 10, 25, 50, 100 µg/mL) were added to the wells and incubated for another 24 hours. Following period of incubation, the cells were examined to determine their viability through the utilization of MTT analysis, and the extracts' cytotoxicity on the cells was assessed.

Electroporation of Plant Extracts

To assess EP's efficacy with various Hypericum perforatum and Rheum ribes extracts concentration (10, 50, 100, 200 µg/mL) and reference drug doxorubicin (1, 10, 50, 100 µg/mL) on A549 cancer

cells, cell solutions at a density of 1 × 106 were prepared and 400 µL of cell solution was placed in each electroporation cuvette (Bio rad, 0.4 cm). Cuvettes were subjected to 8 square wave electrical impulses with pulse widths of 100 µs and frequencies of 1 Hz at electric field strength of 800 V/cm employing a BTX Gemini X2 EP device (Harvard Apparatus, USA). These ECT settings were already optimized for A549 cancer cells in prior experiments (Bendix et al., 2022). Following a 14–15 minute incubation period at room temperature, the cells were seeded into 96-well plates with 10,000 cells per well and left in a carbondioxide incubator for 24 hours. The MTT test was used to evaluate the cells' viability following the period of incubation. ECT is only applied locally to cancer cells, not to healthy cells. Therefore, it was applied only to A549 cancer cells and not to L-929 fibroblast cells.

Cell Viability Assay (MTT)

Cell viability and the cytotoxic potential of the extracts were evaluated using the MTT assay (Kumar et al., 2018). After the incubation periods were completed, the growth medium was removed from the wells, MTT solution dissolved 1/9 ratio in PBS was applied to each well and left to incubate at 37 °C for 4 hours. The solution with MTT was thrown out after 4 hours and 100 µl of Dimethylsulphoxide (DMSO) was administered to each well in order to dissolve the formazan crystals formed during the MTT process. Next, the absorbance was measured using an ELISA reader (Thermo Fisher Scientific, Finland) at a wavelength of 570 nanometers. This test was performed three times for each dose of extract. Due to the influence of light on the MTT, the experiments were conducted in a dark setting. The values of absorbance (AV) calculated from the wells containing Hypericum perforatum, Rheum ribes extracts and doxorubicin solutions were compared with the control absorbance value (AV), and the % viability ratio was determined using the formula below.

% Viability= (Treatment group AV/Control group AV) x100

The IC50 (50% inhibitory concentration) was calculated by plotting the percentage inhibition plotted against the substance’s concentration.

Statistical Analysis

Statistical comparisons among different treatment groups were performed using one-way analysis of variance (ANOVA). In cases where the ANOVA indicated statistically significant differences ($p < 0.05$), post hoc analysis was conducted using Tukey’s Honest Significant Difference (HSD) test to determine pairwise group differences. All statistical analyses were carried out using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). Experiments were independently repeated three times, and the results are presented as the mean ± standard deviation (SD) of the three independent replicates.

RESULTS AND DISCUSSION

Phenolic Substance Contents of Hypericum perforatum and Rheum ribes Plants

In the phenolic compound analysis in our study, extracts of Hypericum perforatum and Rheum ribes were found to contain ascorbic acid, 3,4-Dihydroxybnz acid, trans-p-coumaric acid, 4-Hydroxybenzoic acid, abcisic acid, apigenin, quercetin, kaempferol, myricetin, vanillin, catechol, cinnamic acid, caffeic acid, rosmarinic acid and gallic acid contents (Sun and Shahrajabian, 2023) are shown in Table 1. Phenolic compounds, which are important secondary metabolites, are known to have antioxidative and anticancer properties due to their molecular structure (Neira-Ospina et al., 2024).

Table 1. Phenolic substances content (µg g^{-1}), N/A Non-available

Phenolic Substances	*Hypericum perforatum*	*Rheum ribes*
Ascorbic acid	298,3± 0,89	159,9± 0,13
3,4-Dihydroxybnz acid	N/A	1,4±0,0008
4-Hydroxybenzoic acid	22,3±0,00412	27,92±0,0028
trans-p-coumaric acid	84,6±0,0108	677,4±0,63433
Abcisic acid	31,73±0,0297	N/A
Quercetin	2,544±0,0045	N/A
Apigenin	22354,5±0,92	32,3±0,054
Kaempferol	16,97± 0,0082	N/A
Myricetin	26,4± 0,008	73,8± 0,0538
Catechol	460,3± 0,033	30,842± 0,014
Vanillin	221,4± 0,011	N/A
Caffeic acid	N/A	N/A
Cinnamic acid	23,46± 0,002	8,82± 0,0164
Gallic acid	N/A	N/A
Rosmarinic acid	68,9± 0,00607	25,6± 0,0078

Table 1 shows that the contents of ascorbic acid, quercetin, abcisic acid, kaempferol, apigenin, vanillin, catechol, cinnamic acid and rosmarinic acid in Hypericum perforatum were higher than those of Rheum ribes. In addition, it is seen that the amounts of almost all 16 phenolic substances examined in Hypericum perforatum and Rheum ribes plants in our study are higher than in Arum elongatum Steven plants collected from different regions and used in previous studies (Fawzi Mahomoodally et al., 2023).

Cytotoxic Activity of Extracts and Electrochemotherapy Efficacy

The cytotoxic effect of Rheum ribes and Hypericum perforatum extracts at various doses (0, 5, 25, 50, 100, 200, and 400 g/mL) against both the A549 cancerous cells and fibroblast L-929 cells was

investigated. As a positive control, the chemotherapeutic medication doxorubicin was utilized. Additionally, Rheum ribes and Hypericum perforatum extracts and doxorubicin were applied to cancer cells (A549) using the ECT (extract or drug plus EP) technique, and their activities in ECT were examined. IC50 values for extracts and positive control doxorubicin in chemotherapy and ECT are summarized in Table 2.

Table 2. IC_{50} values of *Hypericum perforatum*, *Rheum ribes* extracts and positive control doxorubicin 24 hours after application in fibroblast (L-929) and cancer (A549) cell lines without and with EP

Plant extracts	**L-929 IC_{50} (µg/mL)**	**A549 IC_{50} (µg/mL)**	**A549 IC_{50} (µg/mL) (with EP)**
Hypericum perforatum	1740.53	241.10	113.4
Rheum ribes	1350.92	297.32	128.51
Doxorubicin (Positive Control)	99.38	16.01	2.21

The cytotoxic activities of the sucbstances applied in vitro to L-929 and A549 cells were evaluated according to their IC50 values. A low IC50 value is associated with strong toxicity, whereas high IC50 value is associated with weak toxicity (Alkış et al., 2021a). Both extracts exhibited almost no cytotoxic effects, with IC50 values higher than 1000 µg/mL for L-929 fibroblast cells (Table 2). However, A549 cancer cells showed very good sensitivity to the cytotoxic activities of Rheum ribes and Hypericum perforatum extracts, with IC50 values of 297.32 and 241.10 µg/mL, respectively. When the cytotoxicity of Rheum ribes and Hypericum perforatum extracts on cancer cells was compared, it was observed that Hypericum perforatum extracts showed a better cytotoxic effect. The possible reason for this is thought to be the higher presence of phenolic compounds with anticancer properties in Hypericum perforatum extracts (Sun and Shahrajabian, 2023) (Table 1).

Several investigations have demonstrated that Rheum ribes and Hypericum perforatum extracts exhibit cytotoxicity in lung cancer similar to the results of our study (Menegazzi et al., 2020; Keser et al., 2020; Taşkonak et al., 2023). Doxorubicin drug, which we used as positive control, showed a much higher cytotoxic effect on A549 cancer cells (Srivastava et al., 2016) (IC50 = 16.01) than Rheum ribes and Hypericum perforatum extracts. Nevertheless, it exhibited a cytotoxic impact on healthy L-929 cells, with an IC50 value of 99.38 µg/mL. Natural products such as Rheum ribes and Hypericum perforatum extracts may be preferred because they are easier to find and have low side effects compared to traditional medicines.

In ECT applications of A549 cancer cells, eight square wave electrical impulse sequences with an intensity of 800 V/cm were used with various doses of Rheum ribes, Hypericum perforatum extracts and doxorubicin. As demonstrated in Table 2, the combined application of doxorubicin or extracts

with EP to A549 cancer cells increased their cytotoxic activity, and decreasing their IC50 values by more than two fold.

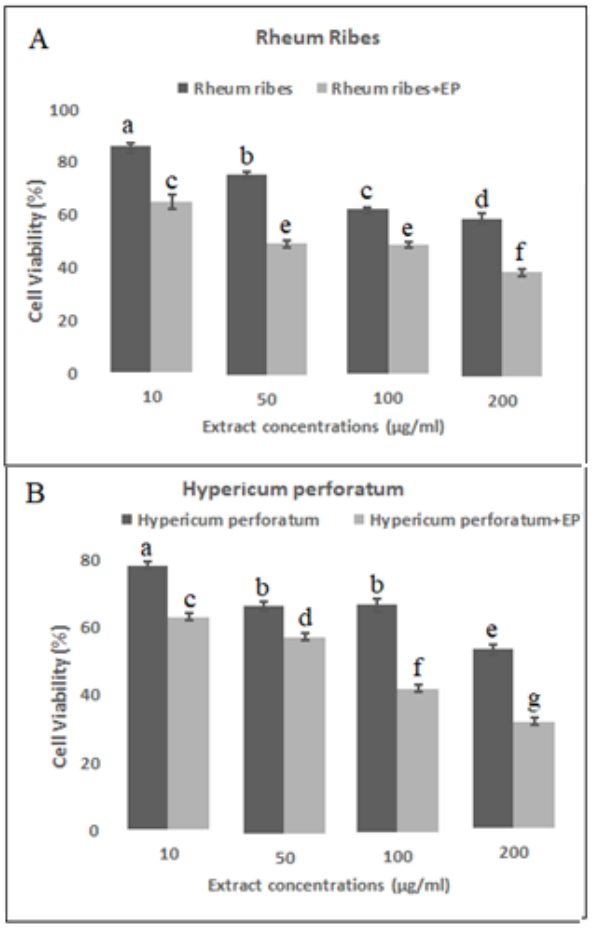

Figure 1. Cell viability percentages of A549 cancer cells after 24 hours exposed to different doses of *Rheum ribes* (A) and *Hypericum perforatum* (B) extracts with or without electroporation (EP). Data was collected from three separate examinations and demonstrated as an average with the standard deviation. There is no statistical difference between columns labeled with the same alphabetic letters ($p>0.05$), but there is a statistical difference between columns labeled with different alphabetic letters ($p<0.05$).

When we compared the extract+EP groups with extract-only groups, it was found that a statistically significant difference existed between them ($p<0.05$) in terms of cell viability percentage at almost all doses (Figure 1). As seen in Figure 1, Rheum ribes or Hypericum perforatum extracts provide the similar activity with 100 µg/mL alone as they do with 10 µg/mL when applied in combination with EP. This shows that effective treatment can be achieved with lower drug doses in ECT, thus reducing the side effects of anticancer agents (Alkis et al., 2022). The electrical pulses utilized may have enhanced the cytotoxic effects of the extracts by raising the permeability and electrical conductivity of A549 cancerous cells (Esmaeili and Friebe, 2019; Bute and Alkis, 2022). Drugs that fight cancer primarily target the DNA within the cell (Doostmohammadi et al., 2024). The interaction between extracts and DNA can lead to DNA fragmentation and cell destruction. ECT may have helped the extracts cause DNA damage by increasing concentrations within the cells. In the literature search, no studies were found on the effect of electroporation on the antiproliferative activity of Rheum ribes and Hypericum perforatum extracts. However, our results are consistent with the results of some previous studies with different plant extracts.

Jeya Shree et al. (2019) investigated the effects of Pulsed Electric Field (PEF) on the cytotoxic activity of Mentha × piperita L. extract in HeLa cells and observed that PEF decreased the IC50 of M. piperita extract from 160 µg/ml to 67.5 µg/ml. They reported that optimal cell death can be attained through PEF treatment, thus lowering the quantity of extract needed for cancer treatment. Choromanska et al. (2020) also state that electroporation significantly enhanced the cytotoxicity of caffeic acid phenethyl ester, a natural phenolic compound, in human melanoma cell lines.

The safety and effectiveness of ECT (substance +EP) against skin and internal organ tumors has been demonstrated in combination of various anticancer medications (Campana et al., 2019; Alkıs, 2021; Alkış et al., 2021b). Mittal et al. (2020) stated that ECT increases the efficacy of curcumin, and that more research is needed for using ECT with curcumin and other medications or vaccinations in the clinic. Similarly, Poompavai et al. (2021) reported that treatment of cancer cells with neem extracts + EP was much more effective than neem extracts alone. The process of EP involves the use of electric pulses to enhance the permeability of the cell membrane. Many experimental studies have revealed that electrical pulses form temporary electropores in the cell, and that when the electric field ends (about 15-20 minutes in in-vitro), their size diminishes and they close again (Lindelauf et al., 2023; Savcı et al., 2022a, 2022b). Thus, the substance or extracts can enter the cell and remain for a longer period of time to exert its effect.

CONCLUSION

The current study findings indicate that both Rheum ribes and Hypericum perforatum extracts are rich in phenolic compounds with antiproliferative properties. While they showed good cytotoxic activity in A549 lung cancer cells, they demonstrated almost no cytotoxic effect in L-929 fibroblast cells. When the cytotoxicity of Rheum ribes and Hypericum perforatum extracts on cancer cells was compared, it was observed that Hypericum perforatum extracts showed a better cytotoxic effect. The cytotoxic activity of both plant extracts increased considerably with EP, and cell viability percentages of extract+EP groups were observed to decrease significantly compared to extract-only groups. These findings suggest that Rheum ribes and Hypericum perforatum extract could possibly help treat lung cancer cells and that EP could significantly increase their antiproliferative activity.

REFERENCES

Akkol, E. K., Koca, U., Pesin, I., & Yilmazer, D. (2011). Evaluation of the wound healing potential ofAchillea biebersteiniiAfan. (Asteraceae) byin vivoexcision and incision models. Evidence-Based Complementary and Alternative Medicine: eCAM, 2011, 1–7. https://doi.org/10.1093/ecam/nep039

Alaca, K., Okumuş, E., Bakkalbaşi, E., & Javidipour, I. (2022). Phytochemicals and antioxidant activities of twelve edible wild plants from Eastern Anatolia, Turkey. Food Science and Technology, 42. https://doi.org/10.1590/fst.18021

Alkis, M. E. (2021). Effects of electroporation on cytotoxicity of 4-aminopyrimidin-2-(1H)-one based ligand and its Cobalt (II) and Ruthenium (II) complexes in MCF-7 cancer cells. Dicle Medical Journal / Dicle Tip Dergisi, 48(3), 498–506. https://doi.org/10.5798/dicletip.988061

Alkis, M. E., Akdag, M. Z., & Kandemir, S. I. (2022). Influence of extremely low-frequency magnetic field on chemotherapy and electrochemotherapy efficacy in human Caco-2 colon cancer cells. Electromagnetic Biology and Medicine, 41(2), 177–183. https://doi.org/10.1080/15368378.2022.2046047

Alkis, M. E., Buldurun, K., Alan, Y., Turan, N., & Altun, A. (2023). Electroporation enhances the anticancer effects of novel Cu(II) and Fe(II) complexes in chemotherapy-resistant glioblastoma cancer cells. Chemistry & Biodiversity, 20(2). https://doi.org/10.1002/cbdv.202200710

Alkış, M. E., Keleştemür, Ü., Alan, Y., Turan, N., & Buldurun, K. (2021a). Cobalt and ruthenium complexes with pyrimidine based schiff base: Synthesis, characterization, anticancer activities and electrochemotherapy efficiency. Journal of Molecular Structure, 1226(129402), 129402. https://doi.org/10.1016/j.molstruc.2020.129402

Alkış, M. E., Turan, N., Alan, Y., Irtegun Kandemir, S., & Buldurun, K. (2021b). Effects of electroporation on anticancer activity of 5-FU and newly synthesized zinc(II) complex in chemotherapy-resistance human brain tumor cells. Medical Oncology (Northwood, London, England), 38(11). https://doi.org/10.1007/s12032-021-01579-7

Magda, M. A., and Nehad, M. G. (2011). Antimicrobial efficacy of Rheum palmatum, Curcumalongaand Alpinia officinarumextracts againstsome pathogenic microorganisms. African Journal of Biotechnology, 10, 12058–12063.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, 117(4), 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Bade, B. C., & Dela Cruz, C. S. (2020). Lung cancer 2020: Epidemiology, etiology, and prevention. Clinics in Chest Medicine, 41(1), 1–24. https://doi.org/10.1016/j.ccm.2019.10.001

Bendix, M. B., Houston, A., Forde, P. F., & Brint, E. (2022). Defining optimal parameters to maximize the effect of electrochemotherapy on lung cancer cells whilst preserving the integrity of immune cells. Bioelectrochemistry (Amsterdam, Netherlands), 148(108257), 108257. https://doi.org/10.1016/j.bioelechem.2022.108257

Bruni, R., & Sacchetti, G. (2009). Factors affecting polyphenol biosynthesis in wild and field grown St. John's Wort (Hypericum perforatum L. Hypericaceae/Guttiferae). Molecules (Basel, Switzerland), 14(2), 682–725. https://doi.org/10.3390/molecules14020682

Bute, B., & Alkis, M. E. (2022). Anticancer activity of methotrexate in electrochemotherapy and electrochemotherapy plus ionizing radiation treatments in human breast cancer cells. Medical Oncology (Northwood, London, England), 40(1). https://doi.org/10.1007/s12032-022-01891-w

Campana, L. G., Miklavčič, D., Bertino, G., Marconato, R., Valpione, S., Imarisio, I., Dieci, M. V., Granziera, E., Cemazar, M., Alaibac, M., & Sersa, G. (2019). Electrochemotherapy of superficial tumors – Current status: Seminars in Oncology, 46(2), 173–191. https://doi.org/10.1053/j.seminoncol.2019.04.002

Chan, W.-J. J., Adiwidjaja, J., McLachlan, A. J., Boddy, A. V., & Harnett, J. E. (2023). Interactions between natural products and cancer treatments: underlying mechanisms and clinical importance. Cancer Chemotherapy and Pharmacology, 91(2), 103–119. https://doi.org/10.1007/s00280-023-04504-z

Choromanska, A., Saczko, J., & Kulbacka, J. (2020). Caffeic acid phenethyl ester assisted by reversible electroporation-in vitro study on human melanoma cells. Pharmaceutics, 12(5), 478. https://doi.org/10.3390/pharmaceutics12050478

Condello, M., D'Avack, G., Spugnini, E. P., & Meschini, S. (2022). Electrochemotherapy: An alternative strategy for improving therapy in drug-resistant SOLID tumors. Cancers, 14(17), 4341. https://doi.org/10.3390/cancers14174341

Crockett, S. L., & Robson, N. K. B. (2011). Taxonomy and chemotaxonomy of the genus Hypericum. Medicinal and Aromatic Plant Science and Biotechnology, 5(Special1), 1–13.

Davis, P. H. (1985). Flora of turkey and the east Aegean islands: Vol.8. Edinburgh University Press.

Doostmohammadi, A., Jooya, H., Ghorbanian, K., Gohari, S., & Dadashpour, M. (2024). Potentials and future perspectives of multi-target drugs in cancer treatment: the next generation anti-cancer agents. Cell Communication and Signaling: CCS, 22(1), 228. https://doi.org/10.1186/s12964-024-01607-9

Erdoğan, M. K., Agca, C. A., & Geçibesler, İ. H. (2020). The antiproliferative potential of isolated emodin and aloe-emodin from Rheum ribes on different cancer cell lines. Biological Diversity and Conservation, 13(2), 160–168. https://doi.org/10.46309/biodicon.2020.753046

Esmaeili, N., & Friebe, M. (2019). Electrochemotherapy: A review of current status, alternative IGP approaches, and future perspectives. Journal of Healthcare Engineering, 2019, 1–11. https://doi.org/10.1155/2019/2784516.

Fawzi Mahomoodally, M., Zengin, G., Roumita, S.-S., Caprioli, G., Mustafa, A. M., Piatti, D., Yıldıztugay, E., Ak, G., Esra Karadağ, A., Khalid, A., Abdalla, A. N., Ibrahim Uba, A., & Demirci, F. (2023). Chemical characterization and multidirectional biological effects of different solvent extracts of arum elongatum: In vitro and in silico approaches. Chemistry & Biodiversity, 20(4), e202201181. https://doi.org/10.1002/cbdv.202201181

Hasani-Ranjbar, S., Nayebi, N., Moradi, L., Mehri, A., Larijani, B., & Abdollahi, M. (2010). The efficacy and safety of herbal medicines used in the treatment of hyperlipidemia; a systematic review. Current Pharmaceutical Design, 16(26), 2935–2947. https://doi.org/10.2174/138161210793176464

Jeya Shree, T., Gowri Sree, V., Poompavai, S., Sieni, E., Sgarbossa, P., Camarillo, I., & Sundararajan, R. (2022). Inhibition of proliferation of HeLa cells by pulsed electric field treatedMentha piperita(mint) extract. IETE Journal of Research, 68(2), 858–868. https://doi.org/10.1080/03772063.2019.1626295

Keser, S., Keser, F., Karatepe, M., Kaygili, O., Tekin, S., Turkoglu, I., Demir, E., Yilmaz, O., Kirbag, S., & Sandal, S. (2020). Bioactive contents, In vitro antiradical, antimicrobial and cytotoxic properties

of rhubarb (Rheum ribes L.) extracts. Natural Product Research, 34(23), 3353–3357. https://doi.org/10.1080/14786419.2018.1560294

Kumar, P., Nagarajan, A., & Uchil, P. D. (2018). Analysis of cell viability by the MTT assay. Cold Spring Harbor Protocols, 2018(6), db.prot095505. https://doi.org/10.1101/pdb.prot095505

Kwiecień, I., Nicosia, N., & Ekiert, H. (2021). Cultivation of Hypericum perforatum (st. John's wort) and biotechnological approaches for improvement of plant raw material quality. In Sustainable Development and Biodiversity (pp. 253–291). Springer International Publishing.

Li F., Wang S.-C., Wang X., Ren Q.-Y., Wang W., Shang G.-W., Zhang L., & Zhang S.-H. (2008). Novel exploration of cathartic pharmacology induced by rhubarb. Zhongguo Zhong yao za zhi [China journal of Chinese materia medica], 33(4), 481–484.

Lindelauf, K. H. K., Baragona, M., Baumann, M., Maessen, R. T. H., & Ritter, A. (2023). Pulse parameters and thresholds for (ir)reversible electroporation on hepatocellular carcinoma cells in vitro. Technology in Cancer Research & Treatment, 22, 153303382211366. https://doi.org/10.1177/15330338221136694

Islam, A., Rahat, I., Anurag, Rejeeth, C., Sharma, D., & Sharma, A. (2025). Recent advcances on plant-based bioengineered nanoparticles using secondary metabolites and their potential in lung cancer management. Journal of Future Foods, 5(1), 1–20. doi:10.1016/j.jfutfo.2024.01.001

Majitol, N., Chor-Wai, L., Jasnie, F., Setina, E.A. (2024). Antioxidant Activity, Total Phenolic, Total Flavonoid and Vitamin C Contents of Hornstedtia havilandii (K. Schum.) K. Schum. from Sabah. Science Letters, 18(2), 16-27. https://doi.org/10.24191/sl.v18i2.25020

Menegazzi, M., Masiello, P., & Novelli, M. (2020). Anti-tumor activity of Hypericum perforatum L. and hyperforin through modulation of inflammatory signaling, ROS generation and proton dynamics. Antioxidants (Basel, Switzerland), 10(1), 18. https://doi.org/10.3390/antiox10010018

Mirmalek, S. A., Azizi, M. A., Jangholi, E., Yadollah-Damavandi, S., Javidi, M. A., Parsa, Y., Parsa, T., Salimi-Tabatabaee, S. A., Ghasemzadeh Kolagar, H., & Alizadeh-Navaei, R. (2015). Cytotoxic and apoptogenic effect of hypericin, the bioactive component of Hypericum perforatum on the MCF-7 human breast cancer cell line. Cancer Cell International, 16(1), 3. https://doi.org/10.1186/s12935-016-0279-4

Mittal, L., Aryal, U. K., Camarillo, I. G., Raman, V., & Sundararajan, R. (2020). Effective electrochemotherapy with curcumin in MDA-MB-231-human, triple negative breast cancer cells: A global proteomics study. Bioelectrochemistry (Amsterdam, Netherlands), 131(107350), 107350. https://doi.org/10.1016/j.bioelechem.2019.107350

Munzuroğlu, Ö., Karataş, F., & Nazmi, G. (2000). A study of the levels of vitamins A, E and C and selenium in rhubarb(Rheum ribes L.). Turk Biyoloji Dergisi [Turkish Journal of Biology], 24(3), 397–404. https://journals.tubitak.gov.tr/biology/vol24/iss3/2

Napoli, E., Siracusa, L., Ruberto, G., Carrubba, A., Lazzara, S., Speciale, A., Cimino, F., Saija, A., & Cristani, M. (2018). Phytochemical profiles, phototoxic and antioxidant properties of eleven Hypericum species – A comparative study. Phytochemistry, 152, 162–173. https://doi.org/10.1016/j.phytochem.2018.05.003

Nehme, Z., Ghandour, F., Hijazi, A., Diab-Assaf, M., & Karaky, R. (2024). Anticancer effect of ethanolic extract of Rheum ribes L. rhizomes: assessment of dose and time-dependent effects in cell-based models. Advances in Traditional Medicine. https://doi.org/10.1007/s13596-024-00772-z

Neira-Ospina, E., Rojas, J., Santa-Gonzalez, G. A., & Garzón, M. A. G. (2024). Phenolic compounds in grapes (genus Vitis): A review of their antioxidant activity, antiproliferative capacity, and cytotoxic effect on colorectal cancer. Journal of Applied Pharmaceutical Science. https://doi.org/10.7324/japs.2024.169381

Öztürk, M., Aydoğmuş-Öztürk, F., Duru, M. E., & Topçu, G. (2007). Antioxidant activity of stem and root extracts of Rhubarb (Rheum ribes): An edible medicinal plant. Food Chemistry, 103(2), 623–630. https://doi.org/10.1016/j.foodchem.2006.09.005

Poompavai, S., Gowri Sree, V., & RamSugitha, B. (2021). Enhanced extraction and improved anticancer activity of neem (Azadirachta indica) leaves by electroporation technique. IETE Journal of Research, 1–11. https://doi.org/10.1080/03772063.2021.1962743

Probst, U., Fuhrmann, I., Beyer, L., & Wiggermann, P. (2018). Electrochemotherapy as a new modality in interventional oncology: A review. Technology in Cancer Research & Treatment, 17, 1533033818785329. https://doi.org/10.1177/1533033818785329

Savcı, A., Buldurun, K., Alkış, M. E., Alan, Y., & Turan, N. (2022a). Synthesis, characterization, antioxidant and anticancer activities of a new Schiff base and its M(II) complexes derived from 5-fluorouracil. Medical Oncology (Northwood, London, England), 39(11). https://doi.org/10.1007/s12032-022-01774-0

Savcı, A., Turan, N., Buldurun, K., Eşref Alkış, M., & Alan, Y. (2022b). Schiff base containing fluorouracil and its M(II) complexes: Synthesis, characterization, cytotoxic and antioxidant activities. Inorganic Chemistry Communications, 143(109780), 109780. https://doi.org/10.1016/j.inoche.2022.109780

Seal, T. (2016). Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, Sonchus arvensis and Oenanthe linearis of North-Eastern region in India. Journal of Applied Pharmaceutical Science, 157–166. https://doi.org/10.7324/japs.2016.60225

Shakya, P., Marslin, G., Siram, K., Beerhues, L., & Franklin, G. (2019). Elicitation as a tool to improve the profiles of high-value secondary metabolites and pharmacological properties of Hypericum perforatum. The Journal of Pharmacy and Pharmacology, 71(1), 70–82. https://doi.org/10.1111/jphp.12743

Smolarz, B., Łukasiewicz, H., Samulak, D., Piekarska, E., Kołaciński, R., & Romanowicz, H. (2025). Lung cancer-epidemiology, pathogenesis, treatment and molecular aspect (review of literature). International Journal of Molecular Sciences, 26(5). https://doi.org/10.3390/ijms26052049

Srivastava, A., Amreddy, N., Babu, A., Panneerselvam, J., Mehta, M., Muralidharan, R., Chen, A., Zhao, Y. D., Razaq, M., Riedinger, N., Kim, H., Liu, S., Wu, S., Abdel-Mageed, A. B., Munshi, A., & Ramesh, R. (2016). Nanosomes carrying doxorubicin exhibit potent anticancer activity against human lung cancer cells. Scientific Reports, 6(1), 38541. https://doi.org/10.1038/srep38541

Strojan, P., Grošelj, A., Serša, G., Plaschke, C. C., Vermorken, J. B., Nuyts, S., de Bree, R., Eisbruch, A., Mendenhall, W. M., Smee, R., & Ferlito, A. (2021). Electrochemotherapy in mucosal cancer of the head and neck: A systematic review. Cancers, 13(6), 1254. https://doi.org/10.3390/cancers13061254

Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants-natural health products for human health. Molecules (Basel, Switzerland), 28(4), 1845. https://doi.org/10.3390/molecules28041845

Taşkonak, B., Aylaz, G., Andac, M., Güven, E., Ozkahraman, B., Perçin, I., & Kılıç Süloğlu, A. (2023). Hypericin-loaded chitosan nanoparticles for enhanced photodynamic therapy in A549 lung cancer cells. BioNanoScience, 13(2), 352–364. https://doi.org/10.1007/s12668-023-01099-w

Thapa, R., Afzal, O., Bhat, A. A., Goyal, A., Alfawaz Altamimi, A. S., Almalki, W. H., Alzarea, S. I., Kazmi, I., Singh, S. K., Dua, K., Thangavelu, L., & Gupta, G. (2023). New horizons in lung cancer management through ATR/CHK1 pathway modulation. Future Medicinal Chemistry, 15(19), 1807–1818. https://doi.org/10.4155/fmc-2023-0164

Tremble, L. F., O'Brien, M. A., Soden, D. M., & Forde, P. F. (2019). Electrochemotherapy with cisplatin increases survival and induces immunogenic responses in murine models of lung cancer and colorectal cancer. Cancer Letters, 442, 475–482. https://doi.org/10.1016/j.canlet.2018.11.015

Venkatadri, R., Gunjan, G., & Rangasamy, A. K. (2011). Antioxidant and anti-cancer potentials ofrheum emodirhizome extracts. Evidence-Based Complementary and Alternative Medicine: eCAM, 2011, 1–9. https://doi.org/10.1093/ecam/neq048

Vieira, A. R., Abar, L., Vingeliene, S., Chan, D. S. M., Aune, D., Navarro-Rosenblatt, D., ... & Norat, T. (2016). Fruits, vegetables and lung cancer risk: a systematic review and meta-analysis. Annals of Oncology, 27(1), 81-96.