ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITY OF GRANNY SMITH APPLE POMACE

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Granny Smith apple pomace was subjected to evaluation as valuable source of antioxidant and anticancer phytochemicals on the basis of its content in phenolic compounds, antioxidant and antiproliferative activity. The total content of phenolics, flavonoids and flavan-3-ols in apple pomace determined spectrophotometrically, was 7.02 mg/g, 0.51 mg/g and 8.80 mg/g. Major phenolics (phenolic acids, flavan-3-ols, flavonoids and dihydrochalcones) in apple pomace were identified and quantified by HPLC. The antioxidant activity of apple pomace on stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) and reactive hydroxyl radicals, was investigated by electron spin resonance (ESR) spectroscopy. The \(IC_{50}^{DPPH}\) and \(IC_{50}^{OH}\) values of Granny Smith apple pomace were 9.51 mg/ml and 29.17 mg/ml, respectively. The antiproliferative activities of apple pomace on cervix epitheloid carcinoma (HeLa), colon adenocarcinoma (HT-29) and breast adenocarcinoma (MCF7) cell lines were determined according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay. The \(IC_{50}^{HeLa}\), \(IC_{50}^{HT-29}\) and \(IC_{50}^{MCF7}\) values of Granny Smith apple pomace were 26.40 mg/ml, 22.47 mg/ml and 21.26 mg/ml, respectively. The significant correlations between antioxidant activities and antiproliferative activities were established (\(p<0.05\)).

KEY WORDS: Apple, Granny Smith, phenolics, antioxidant acivity, ESR, antiproliferative activity

INTRODUCTION

The association between diet rich - fruits and vegetables and a decreased risk of cardiovascular diseases and certain forms of cancer is supported by considerable epidemiological evidence (1, 2). Different studies have shown that free radicals present in the human organism cause oxidative damage to various molecules, such as lipids, proteins...
and nucleic acids, and thus are involved in the initiation phase of degenerative disease. Phenolic and other phytochemical antioxidants found in fruits and vegetables are capable of neutralizing free radicals and may play a major role in the prevention of certain diseases (3). Suggested mechanisms of anticancer effects of phenolics include antioxidant, anti-inflammatory, and antiproliferative activities, as well as their effects on subcellular signaling pathways, induction of cell cycle arrest and apoptosis (4, 5). Apart from their biological properties, phenolic antioxidants are also of interest in the food, cosmetic and pharmaceutical industries, as they can be used as substitutes for synthetic antioxidants (6), since synthetic additives are more and more rejected by consumers because of their toxic properties (7).

Apples are well-known and widespread fruit of the genus Malus (about 25 species) belonging to the family Rosaceae. They make very significant part of the diet in humans and represent a good source of dietary fiber, pectin, potassium, and vitamins A and C (8). Also, apples contain a significant amount of different classes of phenolic compounds such as flavonols (quercetin conjugates), monomeric and oligomeric flavanols, dihydrochalcones, anthocyanidins, p-hydroxycinnamic and p-hydroxybenzoic acids (9, 10). Apples are consumed fresh or in the form of various processed products such as juice, jam, marmalade and dried product. Processing apples into juice has been found to affect phenolic content and antioxidant activity. The conventional apple juice production (straight pressing of apple pulp or pressing after pulp enzyming) results in a juice poor in phenolics and with only 3-10% of the antioxidant activity possessed by the fresh apples. By-products in apple juice processing is apple pomace, which is a rich source of polyphenols, minerals and dietary fibre. The fact that in conventional apple juice production techniques most of the antioxidants remains in the pomace suggests that it can be considered as a source of phenolic antioxidants, which can be extracted and used as dietary supplements or food antioxidants (11).

Our interest is focused on the Granny Smith apple pomace as a potential source of natural phytochemicals. The Granny Smith apples originated in Australia in 1868 from a chance seedling propagated by Maria Smith, where the name "Granny Smith" comes from (12). These apples are a light speckled green in color, though some may have a pink blush. They are crisp, juicy, tart apples which are excellent for both cooking and eating. They are also favored for salads because the slices do not brown as quickly as other varieties (13). The objectives of this study were: (I) to examine phenolic composition of Granny Smith apple pomace using the spectrophotometrical determination of total phenolics, total flavonoids and total flavan-3-ols and individual phenolic compounds by HPLC, (II) to determine the antioxidant activity of Granny Smith apple pomace on stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and reactive hydroxyl radical formed in the Fenton reaction using ESR spectroscopy, (III) to evaluate the antiproliferative activity of Granny Smith apple pomace on HeLa, HT-29 and MCF7 cancer cell growth in vitro, and (IV) to establish correlations between the antioxidant and antiproliferative activities.
EXPERIMENTAL

Chemicals and samples
Methanol and hydrogen peroxide were obtained from „Zorka” Šabac (Serbia). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), Folin-Ciocalteu reagent, vanillin, gallic acid, caffeic acid, chlorogenic acid, (+)-catechin, (-)-epicatechin, phloridzin, rutin were purchased from Sigma Chemical Co. (USA). These chemicals were of analytical reagent grade. Other chemicals and solvents used were of the highest analytical grade.

Granny Smith apples, harvested in the 2005 season, were collected from the Department for fruit growing and viticulture, Faculty of Agriculture, University of Novi Sad.

Pomace preparation
Apples (1 kg) were cleaned by washing, stalks were removed, and the fruits were cut in four pieces and apple pulp was prepared by quick slicing in a domestic food processor (Bosch, Compact Kitchen Machine 4420, Gerlingen-Stuttgart, Germany). Straight pressed apple juice was prepared by immediate pressing of apple pulp. Apple pulp was pressed in a manual fruit and wine press to separate apple juice. The yield of apple pomace was 291.2 g. Moisture of apple pomace was determined by drying a representative 1 g sample in a forced air oven (Sterimatic ST-11, Instrumentaria, Zagreb, Croatia) at 60 °C to the constant mass. Moisture content of apple pomace was 76.7%.

Extraction
Samples of apple pomace (20 g) were extracted at room temperature using an ultrasonic bath, Heidolph DIAX 900 (Heidolph Instruments GmbH, Kelheim, Germany). The extraction was performed three times with different amounts of 80% methanol: 160 ml in 60 min, 80 ml in 60 min, 80 ml in 30 min at room temperature. The total extraction time was 150 min. The obtained three extracts were combined and evaporated to dryness under reduced pressure. The yield, average of triplicate analysis, of apple pomace extract was 2.358 ± 0.099 g.

Total phenolics
Total phenolics in extract were determined spectrophotometrically using the Folin-Ciocalteu reagent and the results are expressed as mg chlorogenic acid equivalents per g dry weight of apple pomace (14).

Total flavonoids
Total flavonoids in extract (expressed as mg rutin per g dry weight of apple pomace) were estimated spectrophotometrically according to Markham (15).

Total flavan-3-ols
Content of total flavan-3-ols in extract was determined spectrophotometrically using the vanillin assay and the results are expressed as mg catechin equivalents per g dry weight of apple pomace (16).
**HPLC analysis**

HPLC analysis was performed using a liquid chromatograph HP1090 (Hewlett-Packard, Avondale, PA, USA) equipped with a diode array detector 79880A DAD (Hewlett-Packard, Avondale, PA, USA). A reversed-phase column, Zorbax SB-C18 (250 × 3.0 mm) with a 5 μm particle size (Agilent, Palo Alto, CA, USA), equipped with pre-column, Zorbax SB-C18 (12.5 × 4.6 mm), was used at a flow-rate of 0.400 ml/min. Solvent gradient was performed by varying the proportion of solvent A (0.1% acetic acid in water) to solvent B (0.1% acetic acid in acetonitrile) as follows: initial 10% B; linear gradient to 20% B in 10 min; linear gradient to 50% B in 30 min; linear gradient to 10% B in 15 min. The set time of recording chromatograms and spectra was 30 min, while the total running time and post-running time were 55 and 5 min, respectively.

The injected volume of sample and standards was 10 μl and it was performed manually. The sample was prepared as methanolic solution with a final concentration of 100 mg/ml. The solution was filtered prior to the injection through 0.45 µm membrane filter (Millipore, Bedford, MA, USA). The column temperature was 23°C. The spectra were acquired in the range of 210-400 nm and the chromatograms plotted at 280/4 nm with reference wavelength 550/100 nm.

Phenolic compounds in samples were identified by matching the retention time and their spectral characteristics against those of standards. The purity of the peaks was determined to ensure the identification. The external standard method was the technique used for quantification. For each compound, a stock solution (concentration of 1 mg/ml) was made by accurately weighing out commercial standard of phenolic compounds followed by dissolution in methanol. Solutions used for calibration were prepared by dilution of the stock solutions. Peak areas from chromatograms were plotted against known concentrations of standards. Equations generated via linear regression were used to establish concentrations of phenolic compounds in the samples.

**DPPH radical assay**

The potential antioxidant activity of extract was assessed on the basis of the scavenging activity of the stable DPPH free radical. Blank probe was obtained by mixing 400 μl 0.4 mM methanolic solution of DPPH and 200 μl of water. The probe contained x μl of 100 mg/ml water solution of extract, (200-x) μl water and 400 μl of 0.4 mM methanolic solution of DPPH radical. The range of the investigated extract concentrations was 2.5-50 mg/ml. After that the mixture was stirred for 2 min and transferred to a quartz flat cell ER-160FT. The ESR spectra were recorded on an ESR spectrometer Bruker 300E (Rheinstetten, Germany) under the following conditions: field modulation 100 kHz, modulation amplitude 0.256 G, receiver gain 2 × 10⁴, time constant 40.96 ms, conversion time 327.68 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.65 GHz, power 7.96 mW, temperature 23 °C.

The antioxidative activity on DPPH radicals (AOA_{DPPH}) was defined as:

\[ \text{AOA}_{\text{DPPH}} = 100 \cdot \frac{(h_o-h_x)}{h_o} \% \]

where \( h_o \) and \( h_x \) are the heights of the second peak in the ESR spectrum of DPPH radicals of the blank and the probe, respectively.
Hydroxyl radical assay

The hydroxyl radicals were generated by Fenton reaction and detected thanks to their ability to form nitroxide adducts from the commonly used DMPO spin trap. Hydroxyl radicals were obtained in the system: 0.2 ml 10mM H₂O₂, 0.2 ml 10 mM FeCl₂ × 4H₂O and 0.2 ml 80mM DMPO as spin trap (blank). The influence of extract on the formation and transformation of hydroxyl radicals was investigated by adding the extract to the Fenton reaction system in the range of concentrations of 5-55 mg/ml. The ESR spectra were recorded after 5 min, with the following spectrometer settings: field modulation 100 kHz, modulation amplitude 0.512 G, receiver gain 5×10⁵, time constant 81.92 ms, conversion time 163.84 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23 °C.

The antioxidative activity on hydroxyl radicals (AOA_{OH}) was defined as:

\[
\text{AOA}_{OH} = 100 \cdot \frac{h_o - h_x}{h_o} \%
\]

where \(h_o\) and \(h_x\) are the heights of the second peak in the ESR spectrum of DMPO-OH spin adduct of the blank and the probe, respectively.

MTT assay

The antiproliferative activity of apple pomace extract was assessed by measuring the inhibition of HeLa (cervix epitheloid carcinoma), MCF7 (breast adenocarcinoma, estrogen receptor-positive) and HT-29 (colon adenocarcinoma) human cancer cell proliferation. Antiproliferative activities were determined according to the MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) colorimetric assay (17). The cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, BRL, UK), supplemented with 10% heat inactivated fetal calf serum (FCS, NIVNS, Serbia), and antibiotics: 100 IU/ml of penicillin and 100 μg/ml of streptomycin (ICN Galenika, Serbia) in Kartel (Kartel, Switzerland) 25 cm³ flasks at 37 °C in atmosphere of 5% CO₂ and high humidity. Cells were maintained in the logarithmic phase of growth and subcultured twice a week using 0.5% trypsin (SIGMA,USA). The cell density (number of cells per unit volume) and the percentage of viable cells were defined as described previously (18). Tumor cells were dispensed into each well of 96-well microtiter plates Corning (Corning, USA) at seeding density of 3×10³ cells per well, in a volume of 180 ml, and preincubated in complete medium at 37 °C for 24h. Extract was added (20 μl/well) to achieve the required final concentrations (10-50 mg/ml). An equal amount of solvent was added to the control wells. Microplates were incubated at 37 °C for 48 h. After incubation, 20 μL of MTT (Sigma, USA), dissolved in DMEM medium solution (5 mg/ml) and filtered through 0.45 μm filter (Sartorius, UK), were added to each well. After a further 3 h incubation, the MTT reaction medium was removed and the purple formazan was dissolved by adding 100 μl of 0.04 mol/l HCl-isopropanol. Cell proliferation was measured by the ability of viable cells to reduce MTT to formazan, whose absorbance can be analyzed photometrically. Absorbance (A) was measured on a microplate reader (Multiscan Ascent, Lab-systems) at 540/620 nm.

The antiproliferative activity (APA) was defined as:
APA = 100·(1-At/Ac) [%]

where $A_t$ and $A_c$ are the absorbances of the test sample and the control, respectively. All measurements were performed in quadruplicate.

RESULTS AND DISCUSSION

The total phenolics, total flavonoids and total flavan-3-ols of Granny Smith apple pomace were determined spectrophotometrically. The Folin-Ciocalteu method is a rapid and widely-used assay to investigate the total phenolic content. The content of total soluble phenolics of Granny Smith apple pomace was expressed as chlorogenic acid equivalent and it was 7.02 mg/g. The Markham method was employed to determine the total flavonoid content of Granny Smith apple pomace. The content of total flavonoids was expressed as rutin equivalent and it was 0.51 mg/g. In order to determine the flavan-3-ols content in apple pomace, the vanillin assay was conducted and (+)-catechin was used as a standard for calibration. The content of flavan-3-ols of Granny Smith apple pomace was 8.80 mg/g. According to the published literature, the differences in the sensitivities to the vanillin assay were observed. Namely, a higher reactivity was observed in the procyandins which are highly polymerised, and the lower reactivity in the catechins which are highly esterified by gallic acid. Besides, epicatechin was more reactive than catechin (19).

Major individual phenolic compounds in apple pomace were identified and quantified by HPLC. A HPLC chromatogram of phenolic compounds in apple pomace is shown in Fig. 1. Phenolic acids (caffeic and chlorogenic acid), flavan-3-ols ((+)-catechin and (-)-epicatechin), flavonols (rutin) and dihydrochalcones (phloridzin) are identified by comparing their retention times and on-line ultraviolet spectra with those of standards. In addition, several other peaks (assigned under the number 6), with retention times ranging from 16.8 min to 21.2 min, had similar spectra as quercetin and were tentatively identified as quercetin-glycosides (20).

![Figure 1. A HPLC chromatogram of phenolic compounds in apple pomace. Peaks: 1 – chlorogenic acid; 2 – caffeic acid; 3 – (+)-catechin; 4 – (-)-epicatechin; 5 – rutin; 6 – quercetin-glycosides; 7 – phloridzin](image-url)
The content of individual phenolic compounds, expressed as mg per dry weight of apple pomace, was as follows: chlorogenic acid, 0.055 mg/g; caffeic acid, 0.038 mg/g; (+)-catechin, 0.054 mg/g; (-)-epicatechin, 0.062 mg/g; phloridzin, 0.009 mg/g; rutin 0.233 mg/g and quercetin-glycosides, 0.237 mg/g. The content of total phenolics in apple pomace determined by Folin-Ciocalteu spectrophotometric method (7.021 mg/g) was higher than the sum of the individual phenolics identified by HPLC (0.688 mg/g). This difference can be explained by the fact that Folin-Ciocalteu method is not an absolute measurement of the amount of phenolics because some other substances such as organic acids, residual sugars, amino acids, proteins and other hydrophilic compounds interfere with this assay. In addition, various phenolic compounds have different responses in Folin-Ciocalteu assay (14, 21).

In this study, the stable DPPH and reactive hydroxyl radicals have been used to investigate antioxidant activity of Granny Smith apple pomace. Fig. 2 shows the influence of the different concentrations of apple pomace on DPPH radical according to ESR data. The typical 1:2:3:2:1 five lines ESR signal of DPPH radical with hyperfine splitting constant $a_N=9.03$ G is inserted in Fig. 2.

![Figure 2](image)

**Figure 2.** Antioxidant activity of different concentrations of apple pomace on DPPH radicals. ESR spectrum of the DPPH radical is shown in the inset.

The influence of the different concentrations of Granny Smith apple pomace on formation and transformation of hydroxyl radical produced in the Fenton reaction is shown in Fig. 3. The inset shows the typical ESR spectrum of DMPO-OH adduct which is characterized by its 1:2:2:1 quartet of lines and hyperfine splitting constant $a_N$ and $a_H=14.9$ G.

Based on the ESR measurements, it can be concluded that Granny Smith apple pomace showed dose-dependent antioxidant activities (AOA$_{DPPH}$ and AOA$_{OH}$). With increasing concentrations of investigated apple pomace, AOA$_{DPPH}$ increased from 0% to 100%, while AOA$_{OH}$ ranged from 0% to 83.37%. The Granny Smith apple pomace has significant antioxidant activity on stable DPPH and reactive hydroxyl radicals. The IC$_{50}$ value is a parameter used to measure antioxidative activity and it is defined as the concentration of sample (expressed in mg dry weight of apple pomace per ml) required for 50% scaven-
ging of DPPH or hydroxyl radicals under experimental condition employed. A smaller IC$_{50}$ value corresponds to a higher antioxidant activity. The IC$_{50}^{\text{DPPH}}$ and IC$_{50}^{\text{OH}}$ values of Granny Smith apple pomace, determined based on antioxidant activities, were 9.51 mg/ml and 29.17 mg/ml, respectively. It was observed that Granny Smith apple pomace was less effective on hydroxyl radical scavenging than in DPPH test.

![Figure 3. Antioxidant activity of different concentrations of apple pomace on hydroxyl radicals generated in the Fenton reaction. The inset shows the ESR spectrum of DMPO-OH adduct](image)

The growth inhibition activity of Granny Smith apple pomace was evaluated in vitro in a panel of 3 histologically different human cancer cell lines: HeLa, MCF7 and HT-29. The results presented herein were obtained by assessing cellular viability by the MTT assay. In viable cells, the yellow tetrazolium salt, MTT, is reduced into a purple formazan by the mitochondrial enzyme, succinate dehydrogenase (SDH) (17). The apple pomace influenced cell growth depending on cell line and dose (Fig. 4).

![Figure 4. Antiproliferative activity of different concentrations of apple pomace on HeLa, HT-29 and MCF7 cell lines](image)
The HeLa cells were inhibited to a high extent. Strong antiproliferative activity toward the growth of HeLa (87.76-95.07%), HT-29 (70.06-88.66%) and MCF7 (64.99-78.01%) cells apple pomace reached at the concentrations ≥ 40 mg/ml. The antiproliferative activities of apple pomace are expressed as the median inhibitory concentration (IC₅₀), with a lower IC₅₀ value signifying a higher antiproliferative activity. The antiproliferative activities of Granny Smith apple pomace on HeLa, HT-29 and MCF7 cell lines expressed as IC₅₀ values were 26.40 mg/ml, 22.47 mg/ml and 21.26 mg/ml.

Components of the fruits have been shown to inhibit cancer cell proliferation in vitro. It is, however, difficult to evaluate the relative importance of individual compounds for the anticancer effects. The protective effect might be due to additive or synergistic actions of several compounds. Enhanced apoptosis and inhibition of cell proliferation was found in human leukemia cell line when treated with quercetin and ellagic acid in combination, compared with the single compounds, proposed to be due to a synergistic effect (22). It is reasonable to assume that phenolic compounds in Granny Smith apple pomace, as strong antioxidants, might influence cell redox state leading to decreased cell proliferation. The inhibition of cancer cell proliferation in experiments of Olsson et al. in HT-29 and MCF7 cell line correlated with vitamin C levels and levels of some carotenoids and anthocyanins present at physiological levels (23).

The relations between the pairs of the accomplished analytical parameters in the presence of different concentrations of apple pomace were determined by correlation analysis. The results are summarized in Table 1.

**Table 1.** Correlation coefficients for analytical parameters in the presence of different concentrations of apple pomace

<table>
<thead>
<tr>
<th>Analytical parameters</th>
<th>AOA₅₀DPPH</th>
<th>AOA₅₀HeLa</th>
<th>APA₅₀HT-29</th>
<th>APA₅₀MCF7</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOA₅₀OH</td>
<td>0.98</td>
<td>0.97</td>
<td>0.91</td>
<td>0.89</td>
</tr>
<tr>
<td>AOA₅₀DPPH</td>
<td></td>
<td>0.99</td>
<td>0.91</td>
<td>0.92</td>
</tr>
<tr>
<td>APA₅₀HeLa</td>
<td></td>
<td></td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>APA₅₀HT-29</td>
<td></td>
<td></td>
<td></td>
<td>0.99</td>
</tr>
</tbody>
</table>

The significant correlations were established between the antioxidant activities on DPPH and hydroxyl radicals, and antiproliferative activities on HeLa, MCF7 and HT-29 cell lines (p<0.05).

The high content of phenolic compounds, antioxidant and antiproliferative activity of Granny Smith apple pomace indicate that it should be regarded as a valuable source of antioxidants that may assist in the prevention of chronic diseases.

**CONCLUSION**

- The total content of phenolics, flavonoids and flavan-3-ols in Granny Smith apple pomace, determined spectrophotometrically, was 7.02 mg/g, 0.51 mg/g and 8.80 mg/g;
Major phenolic phytochemicals in Granny Smith apple pomace (caffeic and chlorogenic acid, (+)-catechin, (-)-epicatechin, phloridzin, rutin and quercetin-glycosides) were identified and quantified by HPLC;
- Employing the ESR spectroscopy, the antioxidant activity of apple pomace on stable DPPH and reactive hydroxyl radicals was established;
- Antioxidant activity of investigated apple pomace increased with increasing concentration;
- The $\text{IC}_{50}^{\text{DPPH}}$ and $\text{IC}_{50}^{\text{OH}}$ values of Granny Smith apple pomace were 9.51 mg/ml and 29.17 mg/ml, respectively;
- The antiproliferative activity of Granny Smith apple pomace was tested by measuring its ability to inhibit proliferation of HeLa, HT-29 and MCF7 cell lines, using MTT assay;
- Apple pomace exhibited high antiproliferative activity on HeLa, HT-29 and MCF7 cell lines;
- The $\text{IC}_{50}^{\text{HeLa}}$, $\text{IC}_{50}^{\text{HT-29}}$ and $\text{IC}_{50}^{\text{MCF7}}$ values of Granny Smith apple pomace were 26.40 mg/ml, 22.47 mg/ml and 21.26 mg/ml, respectively;
- The significant correlations between antioxidant activities on DPPH and hydroxyl radicals and antiproliferative activities on HeLa, MCF7 and HT-29 cell lines were established ($p<0.05$).

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REFERENCES

АНТИОКСИДАТИВНА И АНТИПРОЛИФЕРАТИВНА АКТИВНОСТ ТРОПА ЈАБУКЕ GRANNY SMITH

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У циљу испитивања тропа јабуке сорте Granny Smith као потенцијалног извора различитих фитохемикалија, одређен је садржај фенолних јединиња, као и антиоксидативна и антипролиферативна активност. Садржај укупних фенолних јединиња, фловоноида и флван-3-ола у тропу јабуке, одређени спектрофотометријски, износе 7,02 mg/g, 0,51 mg/g и 8,80 mg/g. Најзаступљенија фенолна јединиња (фенолне киселине, флван-3-оли, фловоноиди и дицирхалкони) у тропу јабуке су идентификована и квантитативно одређена HPLC методом. Антиоксидативна активност тропа јабуке на стабилне 1,1-дифенил-2-пикрилхидразил (DPPH) и реактивне хидроксил радикале испитања је електрон спин резонантном (ESR) спектроскопијом. IC50 DPPH i IC50 OH вредности тропа јабуке Granny Smith износе 9.51 mg/ml и 29.17 mg/ml. Антипролиферативна активност испитања је на одабране линије туморских ћелија: хуманог епителног карцинома цервикса (HeLa), хуманог аденоокарцинома дебелог црева (HT-29) и хуманог аденоокарцинмом дојке (MCF7) применом MTT (3-(4,5-диметилтиазол-2-ил)-2,5-дифенилтетразолиум бромид) теста. IC50 HeLa, IC50 HT-29 и IC50 MCF7 вредности тропа јабуке Granny Smith износе 26.40 mg/ml, 22.47 mg/ml и 21.26 mg/ml. Значајна корелација утврђена је између антиоксидативних и антипролиферативних активности (p<0.05).

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