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# Cytotoxicity of Some Edible Plants toward Ehrlich Ascites Carcinoma Cells

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## ABSTRACT
The goal of this study was to perform some contribution into the knowledge on the antitumor ability of plants, traditionally used in folk medicine in Armenia. The water and ethanol extracts of dried leaves of grape, sorrel and sea buckthorn, rose petals, melilot, pellicles of walnut kernels were prepared. Their ability to suppress the growth of cultivated mice Ehrlich Ascites Carcinoma (EAC) cells and Mice Peripheral Blood Leukocytes (MBL) was screened. The ethanol extracts of rose petals, grape and sorrel leaves were fractionated by gel-filtration. The extracts and obtained fractions were characterized by antioxidant activity, qualitative chemical analysis, thin layer chromatography and UV-Vis absorbance. The $IC_{50}$ values for inhibition of EAC cells' growth by ethanol extracts of the three plants were evaluated and appeared in nano-gram range. $IC_{50}$ values for DPPH and ABTS radicals' scavenging by ethanol extracts were evaluated. The antioxidant activities of the three studied extracts of plants were in the order: rose petals>grape leaves>sorrel leaves. The most of phenol glycosides from the three plants with strong cytotoxicity toward EAC cells and practically no effect on the MBLs, possessed relatively lower antioxidant activity. The strong toxicity of the extract of sorrel leaves as a whole and of its phenol glycoside and anthracene derivative fractions to EAC cells and low action on MBLs underlined the preference of sorrel leaves as an anticancer remedy. Based on the obtained results, it can be assumed that the extracts of the studied plants and their constituents may be recommended as sources for anticancer drugs.

**Key words:** Anticancer and antioxidant activities, Ehrlich ascites carcinoma cells, grape leaves (*Vitis vinifera*), rose petals (*Rosa damascena*), sorrel leaves (*Rumex crispus*)

## INTRODUCTION
Lots of cancer chemotherapeutics which are currently in usage, affect the normal cells of patients strongly. They have hepatotoxic, nephrotoxic, cardiotoxic, etc., side effects on normal cells and become serious medical problems. This induced scientists to search for new medicaments from various sources, for instance, from medicinal plants. There is a growing trend for herbal drugs because of low toxicity and high medical effectiveness of plant extracts. The anticancer features of plants have been recognized for centuries: millions of plant products exist possessing such properties (Desai *et al*., 2008). Recently, 52 and 36 plants with tumoricidal character have been presented in the reviews of Kaur *et al*. (2011) and Nirmala *et al*. (2011), respectively.

Medicine was an inseparable part of ancient Armenian culture and its roots come from deep in the past. Armenia has a rich history in the field of natural medicine, particularly, in phytotherapy. Since the V century BC, some medical plants were disseminated in Europe from here

(Vardanian, 1982). Herbal medicine developed thanks to medieval Armenian scholars Mkhitar Heratsi, Amirdovlat Amasiatsi, Grigor Magistros and many others (Torosian, 2006). Several dietary plants widespread in Armenia are used in folk medicine. For example, melilot (*Melilotus officialis*), various parts of sea buckthorn (*Hippophae rhamnoides* L.), walnut (*Juglans regia* L.), grape (*Vitis vinifera* L.), sorrel (*Rumex* L.) and rose (*Rosa damascene*), etc., show different pharmacological effects and are used in traditional and current medicine for treatment of various health disorders. However, at present, little information is available about their anticancer properties.

Earlier the ability of aqueous extracts of 27 plants of Armenian Highland origin to inhibit the purified preparations of dipeptidyl peptidase IV and adenosine deaminase-the enzymes, activities of which are elevated at diabetes mellitus, were tested *in vitro* (Mardanyan *et al*., 2011). The obtained results have shown that such plants, as: Blackberry, melilot, oregano, St. John's wort, sea-buckthorn leaves, etc., can be used in combination with the usual antidiabetic drugs to enhance their effectiveness at treatment of diabetes mellitus.

In the present study, the cytotoxicity of the extracts of leaves of grape, sea buckthorn and sorrel, rose petals, pellicles of walnut kernels and aboveground parts of melilot toward cancer cells (mice Ehrlich ascites carcinoma, EAC) was scanned. This study was compared with their influence on the viability of normal cells (leukocytes of mice peripheral blood, MBL). The ethanol extracts of petals of rose, leaves of grape and sorrel were subjected to fractionation by gel-filtration. The extracts and the obtained fractions were characterized by chemical analysis, thin layer chromatography, UV-VIS absorbance and antioxidant activities. The described in the present article results signify the potential of studied plants as sources of anticancer therapeutic agents. The findings also validate their use in traditional medicine.

## MATERIALS AND METHODS

**Reagents and equipments:** 2, 2-diphenyl-1-picrylhydrazyl (DPPH); 2, 2 azobis-(3-ethylbenzothiozoline-6-sulphonic acid) (ABTS); Cell cultivation RPMI-1640 medium and additions were purchased from Sigma Ltd, USA; G-25 and LH-20 Sephadex-from Pharmacia Biotech, Upsala, Sweden. Other chemicals were of the highest purity.

UV-Vis absorption spectra were recorded on Specord M-40 (Germany) spectrophotometer. For monitoring the cell growth and for DNA comet assay the light and fluorescence microscopes model BH- 2RFCA, Olympus, with a digital camera, model c35AD-4, Olympus were used.

**Plant material:** The leaves of grape (*Vitis vinifera*), sorrel (*Rumex crispus*) and sea buckthorn (*Hippophae rhamnoides*), rose (*Rosa damascena*), melilot (*Melilotus officinalis*) and the walnut (*Juglans regia*) were collected from the flanks of Aragats Mountain and in Ararat valley of Armenia. A voucher specimen has been deposited at the herbarium of the Botanical Department of Yerevan State University (Dr. Narine Zaqaryan). The dried in shade plant material was grinded and the 10% (w/v) extracts were prepared in bi-distilled water (30 min in boiling water bath) and in 70% ethanol (72 h at ambient temperature). The extracts were filtered through a sterile cheese cloth, dried at 37°C to constant weight and stored at -18°C until using.

**Fractionation of plant extracts:** The appropriate amount of the dried extract, usually, 1-3 mg, was dissolved in 2 mL of 70% ethanol and subjected to gel-filtration on LH-20 Sephadex containing column (2×40 cm), equilibrated with 20% ethanol. The fractions were eluted with increasing concentration of ethanol up to 70%. The fractions containing the mixture of different compounds were subjected to gel-filtration on G-25 Sephadex column, equilibrated with 20% ethanol and

eluted with an appropriate concentration of ethanol. The received fractions were dried to constant weight by evaporation.

**Identification of chemical constituents of plant extracts:** The constituents of the extracts and the fractions were characterized by qualitative chemical analysis (Grinkewich and Safronich, 1983). The appearance of characteristic colors in the following specific reactions were used: Bright yellow-for flavonoids (Fl) in basic medium (5% ethanol solution of $AlCl_3$ was also used); dark violet-for phenol glycosides (PhG) in the presence of $FeSO_4$ crystals; black-green-for tannins at reaction with 1% iron ammonium alums; pink-red-for anthracene derivatives (AD)-in the presence of 5% ethanol solution of sodium hydroxide; bright red-cherry-for coumarins (Cm) at adding of Pauli's reagent after boiling in basic medium. Then, for the detection of phloroglucides (Phlg)-Pauli's reagent; alkaloids-the Dragendorff's reagent; carbohydrates-the Benedict's test and cardio glycosides-reactions of Legal and Keller-Kiliani were used. Saponins presence was checked by the formation of a frothing after boiling and vigorous mixing.

**Thin-layer chromatography:** The constituents of the extracts and fractions, dissolved in different concentrations of ethanol (depending on the compound type), were characterized by TLC analysis on silica gel sheets (glass support, Fluke), in the appropriate solvent system: For PhG-EtOAc/$CH_2O_2$/$H_2O$ (3/2/1), revealing by 1% solution of $FeCl_3$ in ethanol; for AD-EtOAc/MeOH/$H_2O$ (100/17/13), revealing by 5% solution of KOH in methanol; for Fl-EtOAc/$CH_2O_2$/$H_2O$ (8/3/1), revealing by 1% solution of $AlCl_3$ in ethanol; for Cm-EtOAc/MeOH/$H_2O$ (3/1/1), revealing by 10% $H_2SO_4$ followed by heating until the appearance of spots.

**Antioxidant assays:** The antioxidant activities of the extracts and fractions were estimated by two methods: DPPH and ABTS radicals scavenging effects. Each sample was dissolved in DMSO at concentration 10 mg $mL^{-1}$ and diluted in 70% ethanol to 1 mg $mL^{-1}$. Then, definite concentrations in 70% ethanol were prepared right before duplicate analysis for antioxidant capacity:

- The DPPH assay was done according to the method of Brand-Williams *et al.* (1995). Briefly: The methanol solution of DPPH, with absorbance of about 1.05 (±0.02) at 517 nm was prepared daily. A 1 mL aliquot of this solution was mixed with 100 μL of a sample at appropriate concentration. The solution in the test tube was shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance at 517 nm was measured
- The ABTS Radical scavenging activity was assayed according to Re *et al.* (1999): 7 mM ABTS solution reacted with 2.45 mM potassium persulfate, kept overnight in the dark and diluted with 0.05 M phosphate buffer, pH 7.4 to get an absorbance of about 0.800 (±0.02) at 743 nm. A 1.0 mL of this solution was mixed with 100 μL of a sample at appropriate concentration and in 6 min the absorbance at 743 nm was measured

Along with the analysis of samples, ascorbic acid solution was used as a standard. At both assays, the control mixtures contained 70% ethanol instead of the test solution and the percentage scavenging activity of the plant sample (and of the standard, ascorbic acid) solution was estimated using the following equation:

$$\text{Scavenging effect (\%)} = [(\text{control absorbance-sample absorbance})/(\text{control absorbance})]\times 100$$

The scavenging activity of a plant sample was expressed as the concentration of ascorbic acid with the activity, equal to the activity of sample. $IC_{50}$ value was determined as the concentration of a plant preparation in μg $mL^{-1}$ with ability to scavenge 50% of free radicals.

**Primary cell culture of mice ehrlich ascites carcinoma (EAC) cells:** The line of EAC cells was supported by re-inoculation into the peritoneal cavity of laboratory mice (Harutyunyan, 2010). On the 7th day after infection, the ascitic fluid, containing EAC cells, was taken from animals under aseptic conditions. Cells were washed twice with the sterile Phosphate Buffered Saline (PBS), pH 7.2 and suspended in the RPMI-1640 medium, containing 10% fetal bovine serum and penicillin (50 IU $mL^{-1}$). The concentration of cells were adjusted to $0.5\times10^6$ cells $mL^{-1}$ and cultivated during 5 days in the humidified chamber with 5% $CO_2$ at 37°C.

**Primary cell culture of mouse peripheral blood leucocytes:** The blood was taken from mouse by cardiac puncture with sterile heparinized (20-50 units $mL^{-1}$) syringe. After centrifugation of whole blood at 380×g during 10 min, the rich of leucocytes fraction was collected from buffy coat. Leucocytes were washed three times in PBS and suspended in RPMI-1640 medium containing fetal bovine serum (15%), penicillin (50 IU $mL^{-1}$) and phytohemagglutinin-M (200 μg $mL^{-1}$) (Miller and Enders, 1968). The final concentration and the cultivation conditions of leucocytes were as for the EAC cells.

**Viability of cells:** To investigate the cytotoxicity of plant extracts and their constituents toward EAC and MBL cells, a definite amount of plant sample was dissolved in DMSO and added to the cultivation medium up to the appropriate concentration. The amount of DMSO in the medium did not exceed 1%. Special test ensured the absence of affectivity of this DMSO concentration. The concentration of cells was measured daily by light microscopy using the counting chamber. The amount of viable cells was determined by trypan blue exclusion test as percentage of the total amount of cells (Strober, 2001). DNA Comet assay by Ostling and Johanson (1984) was used for genotoxisity qualifying, as it was done previously (Movsisyan *et al*., 2012).

**Statistical analyses:** The obtained data were analyzed using the statistical software InStat, version 3 for Windows (GraphPad Software, Inc., San Diego, CA, USA). All results are the averages of three independent analyses. Data are presented as Mean±S.E.

## RESULTS

**Screening of plant extract influence on the growth of cultured EAC cells:** The EAC cells were incubated in the medium containing water or ethanol extract of either Grape Leaves, (GL), Sorrel Leaves (SL), Sea Buckthorn Leaves (SBL), Rose Petals (RP), above ground part of Melilot (M) and Pellicles of Walnut Kernels (WKP). The effects of the water extracts of these plants were not clearly pronounced. In Fig. 1, the 3 day effects of 1% ethanol extracts of the studied six plants on the EAC cells' growth are shown. Here, the amount of alive cells in the presence of the extract is expressed as percentage of the amount in control sample. One can see that in 2-3 days, the studied extracts inhibited the growth of EAC cells by 50-100 percent compared with that in the control sample.

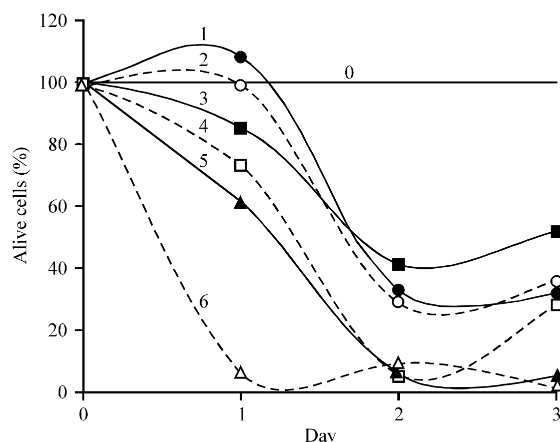


Fig. 1: Inhibition of growth of ehrlich ascites carcinoma cells by 1% ethanol extracts of six edible plants. The amount of alive cells in the presence of plant extract is expressed as percentage of the amount in the control sample: (0) Control, without plant extract, (1) Leaves of grape, (2) Pellicles of walnut kernels, (3) Petals of rose, (4) Leaves of sorrel, (5) Melilot and (6) Leaves of sea buckthorn

Table 1: Chemical composition of plant extracts[a]

| Plant | Flavonoids | Phenol glycosides | Tannins | Anthracene derivatives | Coumarins | Phloroglucides | Cardio glycosides | Alkaloids | Saponins |
|---|---|---|---|---|---|---|---|---|---|
| Leaves of sorrel | +++ | + | ++ | +++ | ++ | ++ | No[b] | + | No |
| Leaves of grape | +++ | ++ | ++ | No | ++ | ++ | No | + | No |
| Pellicles of walnut kernels | ++ | +++ | +++ | No | Neg[c] | +++ | +++ | +++ | No |
| Melilot | +++ | No | + | No | +++ | No | No | + | No |
| Rose petals | ++ | +++ | ++ | No | ++ | +++ | ++ | + | No |
| Leaves of sea buckthorn | ++ | +++ | ++ | No | ++ | ++ | ++ | No | No |

[a]Comparative quantity evaluated by quality analysis in the identical aliquots of the extracts: +++>++>+, [b]No-not observed, [c]Negligible

**Chemical composition of plants:** The qualitative chemical analysis of the water and ethanol extracts of GL, SL, SBL, RP, M and WKP revealed the existence in them of the compounds, presented in Table 1. From these data we can see that in WKP the most of the analyzed chemicals were obtained: Tannins, phenol glycosides, cardio glycosides, phloroglucides, alkaloids but not coumarins, the flavonoids were found nearly in all of the studied extracts. They are the main chemical compounds in GL. RP were rich in phenol glycosides and phloroglucides, but anthracene derivatives were found only in SL. Saponins were not found in any of the studied plants.

**Fractionation of extracts:** A more detailed investigation was continued with ethanol extracts of GL, SL and RP, regularly used in Armenian cuisine. These three extracts were fractionated by gel-filtration on columns with LH-20 and G-25 Sephadex equilibrated with 20% ethanol solution and eluted by increasing concentration of ethanol. These procedures resulted in receiving of several fractions, among them-coumarin, phenol glycoside and anthracene derivative fractions from the extract of SL; phloroglucide, 2 coumarin and 2 phenol glycoside fractions from the extract of GL, 2 coumarin and three phenol glycoside fractions from RP.

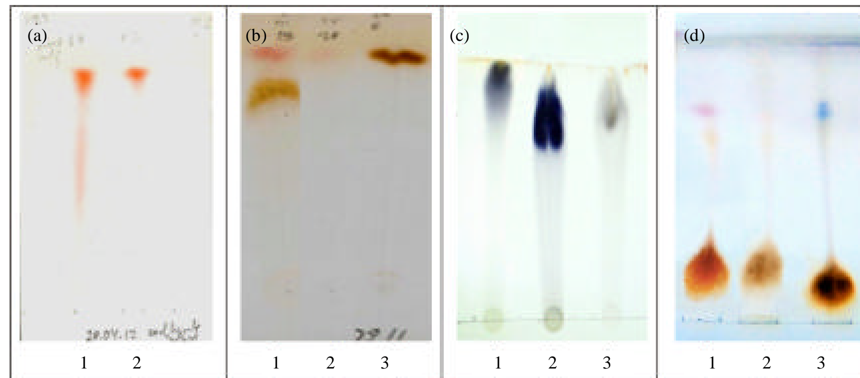


Fig. 2(a-d): Thin-layer chromatography pictures of some plant preparations. (a) The extract of sorrel leaves (1) and isolated from it anthracene derivative (2) in EtOAc/MeOH/$H_2O$ (100/17/13) solvent system. (b) The extract of sorrel leaves (1), isolated from it fraction of flavonoids (2) and quercetin as a control (3) in EtOAc/$CH_2O_2$/$H_2O$ (8/3/1) solvent system. (c) The phenol glycoside fractions from extracts of rose petals (1), grape leaves (2) and sorrel leaves (3) in EtOAc/$CH_2O_2$/$H_2O$ (3/2/1) solvent system. (d) The coumarin fractions from extracts of rose petals (1), sorrel leaves (2) and grape leaves (3) in EtOAc/MeOH/$H_2O$ (3/1/1) solvent system

**Thin layer chromatography:** In Fig. 2a and b, the Thin Layer Chromatography (TLC) pictures of sorrel extract and of isolated from it AD and Fl fractions are presented. The absence of tails and of residues in the initial points of the traces 2 evidenced the purity of isolated AD and Fl fractions. In Fig. 2c, the TLC pictures of PhG fractions from GL, RP and SL (traces 1, 2 and 3, respectively) are presented. The characteristic colors of spots prove the results of chemical analysis (Table 1). In Fig. 2d, the TLC picture of Cms from extracts of RP, SL and GL (traces 1, 2 and 3, respectively) are shown. The red-brown color of low-mobility spots manifests that these fractions contain a significant amount of sugar. The glycoside group contamination in these fractions along with Cms was proved also by the qualitative analysis for carbohydrides, as well as by appearance of specific lines in the region of chemical shifts between 3 and 4 ppm in NMR spectra (Homans *et al*., 1987) (not shown). These fractions were further subjected to phase separation in the $H_2O$/EtOAc system which resulted in obtaining glycoside and coumarin fractions in the water and the ethyl acetate phases, respectively. The following investigation demonstrated the cytotoxicity of both of the separated fractions toward EAC cells.

**Optical absorbance of isolated constituents:** In Fig. 3, the spectra of optical absorbance in UV-VIS region of fractions, isolated from plant extracts upon gel-filtration procedures, are presented. In general, the characters of the registered spectra reaffirm the results of chemical and TLC identifications. Two coumarin fractions received both from RP and GL differed by the wavelengths of absorption peaks. The spectra of Cm from SL and Cm1 from RP are similar, with absorption peaks at 270 nm (Fig. 3a, curve 1). The absorption peak of Cm2 from RP is at 315 nm (Fig. 3a, curve 2). In spectra of Cm1 and Cm2 from GL the absorption peaks are shifted to lower wavelength (Fig. 3b, curves 1 and 2). Based on the publication of Kim *et al*. (2003), we can conclude, that only Cm2 from RP with $\lambda$max >300 nm is in monomer form, all the other Cms which

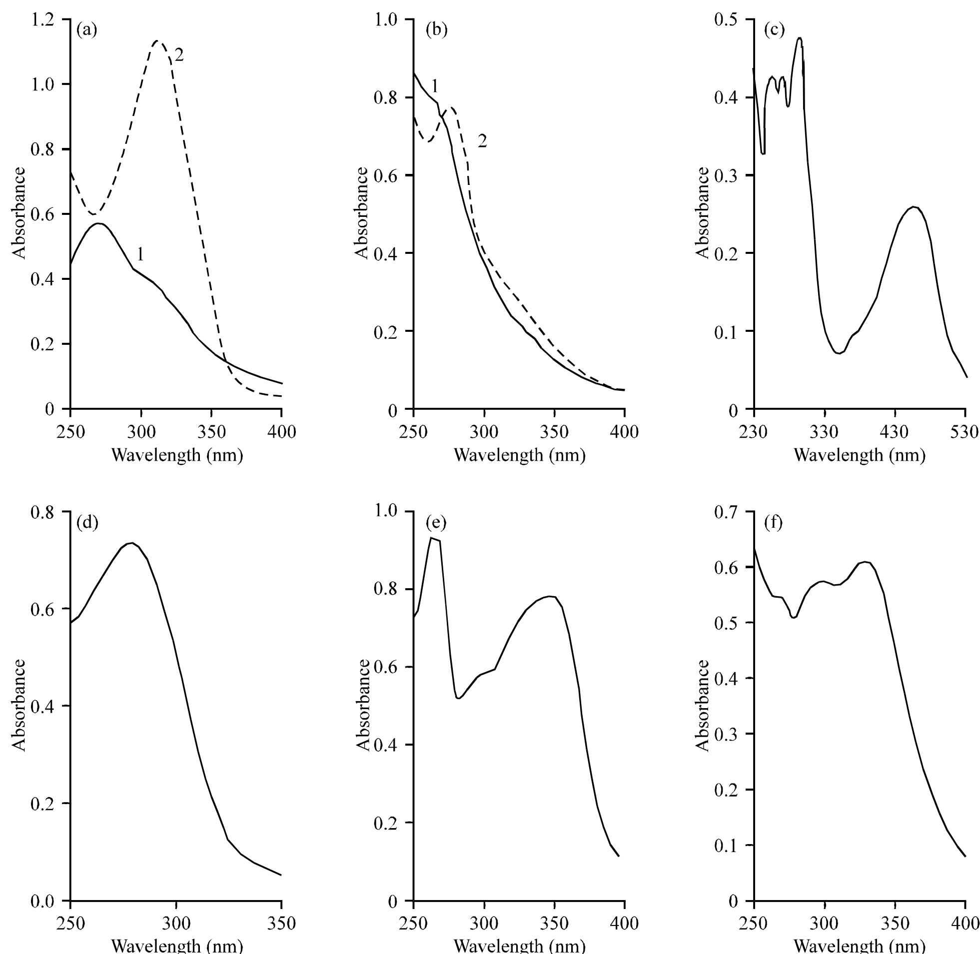


Fig. 3(a-f): Optical absorbance spectra of fractions, isolated from plant extracts by gel-filtration. (a) Coumarin from sorrel leaves, coumarin1 from rose petals (1) and coumarin2 from rose petals (2), (b) Coumarin1 (1) and coumarin2 (2) from grape leaves, (c) Anthracene derivative from sorrel leaves, (d) Phloroglucide from grape leaves, (e) Phenol glycosides and (f) Flavonoids. The last two compounds existed in all of the studied extracts (Table 1)

λmax <300 nm, with high probability are in the dimeric form. The spectrum of anthracene derivative from SL (Fig. 3c) allows suggesting that it has a structure of emodin.

**Antioxidant properties:** In Table 2, the antioxidant activities of RP, SL and GL extracts and their constituents at concentration 1 mg $mL^{-1}$, evaluated by DPPH and ABTS methods, are shown. The activities of plant preparatons determined by the both methods are equal to the activity of ascorbic acid at concentration intervals: 1-3.3 μg $mL^{-1}$ for Cms; 45-100 μg $mL^{-1}$ for Phlg of GL; 20-200 μg $mL^{-1}$ for PhGs. The AD from SL scavenged only ABTS radicals with the activity corresponding to the activity of ascorbic acid at concentration 14 μg $mL^{-1}$. It can be noted that the values of antioxidant activities of compounds belonging to the same chemical type are different. For instance, the different fractions of PhG from RP, eluted from Sephadex columns by different concentrations of ethanol, differ from each other up to 8 times. It manifests that the obtained

Table 2: Antioxidant activity of some plant preparations[a]

| | $\mu g\ mL^{-1}$ (asc. acid) | |
|---|---|---|
| Name of preparation | DPPH | ABTS |
| **Sorrel leaves** | | |
| Extract | 3.7 | 9.1 |
| Coumarin | 1.25 | 1.1 |
| Phenol glycoside | 95 | 77.5 |
| Anthracene derivative | 0 | 14 |
| **Grape leaves** | | |
| Extract | 19 | 39 |
| Coumarin 1 | 3.25 | 0.9 |
| Coumarin 2 | 2.5 | 2.9 |
| Phenol glycoside 1 | 45 | 60 |
| Phenol glycoside 2 | 102 | 200 |
| Phloroglucide | 49 | 92.5 |
| **Rose petals** | | |
| Extract | 40 | 59 |
| Coumarin 1 | 2.4 | 1.9 |
| Coumarin 2 | 1.25 | 1.5 |
| Phenol glycoside 1 | 19 | 20.5 |
| Phenol glycoside 2 | 42.5 | 85 |
| Phenol glycoside 3 | 176 | 76.3 |

[a]Scavenging activity of 1 mg $mL^{-1}$ plant preparation is expressed in equivalent of ascorbic acid (standard) in $\mu g\ mL^{-1}$ possessing this activity

phenol glycosides possess different molecular structures. The data in Table 2 evidence that the antioxidant activities of the extracts of the three studied plants at concentration 1 mg $mL^{-1}$ are in the order: RP>GL>SL. Their $IC_{50}$ values were evaluated from the concentration dependencies of antioxidant activities of the extracts. The data for DPPH and ABTS radicals scavenging were, respectively: 9.03±0.27 and 2.79±0.5 $\mu g\ mL^{-1}$ for RP; 17.85±0.52 and 3.79±0.09 $\mu g\ mL^{-1}$ for GL; and 115.94±1.09 and 14.38±0.14 $\mu g\ mL^{-1}$ for SL. These values reaffirm the order of reducing ability of the extracts.

**Action of plant preparations on the viability of EAC and MBL cells:** In Fig. 4, the effects of ethanol extracts of SL, RP and GL and of their constituents at concentration 200 $\mu g\ mL^{-1}$ on the viability of EAC cells and MBLs are shown. It is evident that the extracts of the three plants inhibited the growth of cells differently. Unlike the extracts of RP and GL, the extract of SL inhibited EAC cells more effectively, than MBLs.

In this figure, one can see that among coumarin fractions, Cm2 of RP inhibited EAC cells most effectively. The inhibiting ability of Cm from SL and Cm1 from RP on EAC cells amounted to 40-55%, while they practically had no influence on MBLs. Since two Cms from GL manifested similar toxicity to both EAC and MBL cells, we performed DNA-comet assay (Fig. 5) to research the mechanism of killing the cells by Cm1 from GL. The obtained result evidenced in favor of apoptotic mechanism.

PhG2 from GL and PhG3 from RP possessed some cytotoxicity toward EAC cells (Fig. 4), but almost did not influence MBLs, while PhG from SL, PhG1 from GL, PhG1 and PhG2 from RP possessed rather strong cytotoxicity toward EAC cells and practically had no effect on MBLs. The anthracene derivative fraction from the ethanol extract of SL also demonstrated a relatively mild toxicity toward normal cells and strong toxicity to EAC cells.

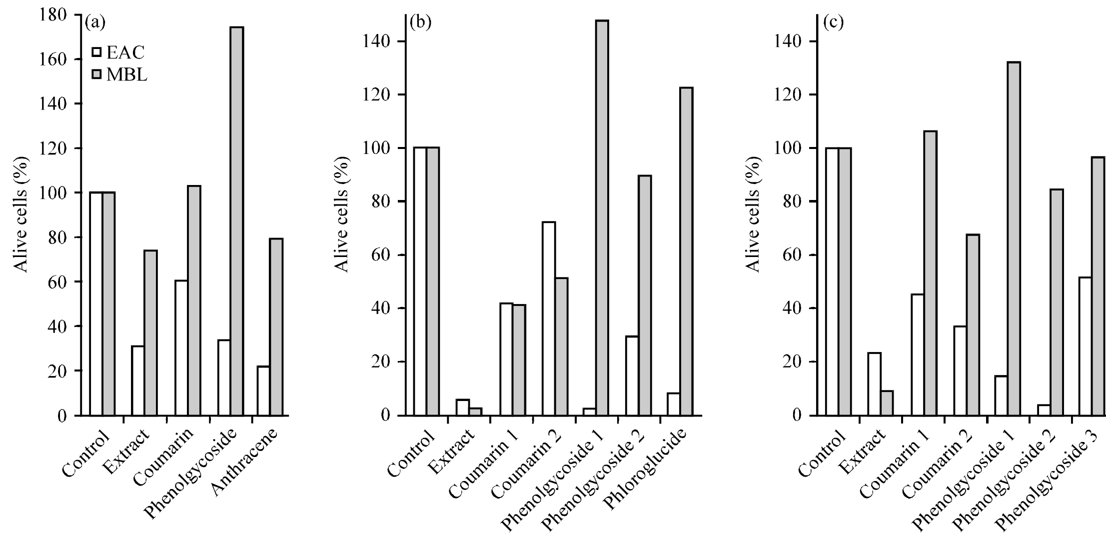


Fig. 4(a-c): Influence of plant preparations on the viability of ehrlich ascites carcinoma cells and mice blood leukocytes. The cells were cultivated two days in the presence of 200 μg $mL^{-1}$ of indicated plant preparation. The amount of alive cells in each sample is expressed as percentage of the amount in the control sample (a) Sorrel leaves, (b) Grape leaves and (c) Rose patels

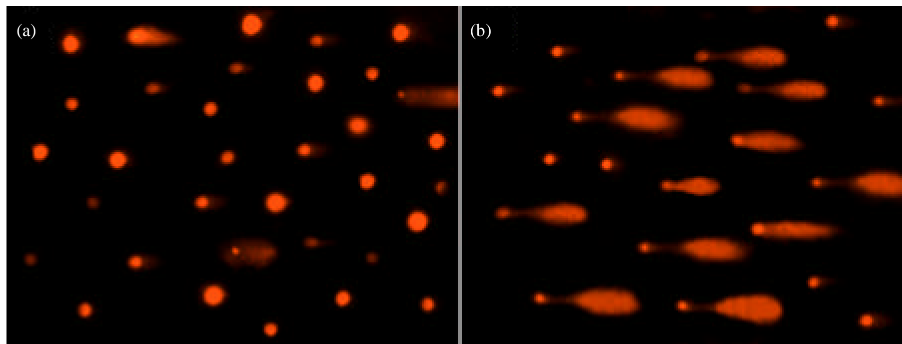


Fig. 5(a-b): DNA comet pictures of ehrlich ascites carcinoma cells after 24 h cultivation (a) In the absence and (b) In presence of coumarin from grape leaves

The concentration dependencies of cytotoxicity of SL, GL and RP extracts toward EAC cells and MBLs were studied. The results elucidated that the extracts of these plants at concentration 4 μg $mL^{-1}$ inhibited the growth of EAC cells by 71, 73 and 79%, respectively, leaving the viability of MBLs at 95-100% (not shown). This confirmed the high toxicity of the studied extracts toward cancer cells and the low toxicity toward normal cells.

The $IC_{50}$ values of the three plant extracts for inhibiting the growth of MBLs, revealed from the concentration dependencies, were in the order: SL>GL>RP (25±10 and 1.03±0.2 mg $mL^{-1}$ and 73±3 μg $mL^{-1}$, respectively). This order is consistent with the decrease of their antioxidant activities: RP>GL>SL (Table 2). $IC_{50}$ values relating to the inhibition of growth of EAC cells for the extracts of GL and RP (80±5 and 15±2 ng $mL^{-1}$, respectively) were of the same order; however, $IC_{50}$ for the extract of SL (0.1±0.04 ng $mL^{-1}$) was lower by three orders than that for GL.

Table 3: $IC_{50}$ values for inhibiting the growth of ehrlich ascites carcinoma cells by plant fractions

| Fraction | $IC_{50}$±s.e (μg $mL^{-1}$) |
|---|---|
| Phenol glycoside -1 of rose petals | 0.15±0.020 |
| Phenol glycoside -2 of rose petals | 0.46±0.150 |
| Phenol glycoside -2 of grape leaves | 30.00±2.100 |
| Phloroglucide of grape leaves | 25.00±2.200 |
| Phenol glycoside of sorrel leaves | 0.93±0.200 |
| Anthracene derivative of sorrel leaves | 0.037±0.01 |

In Table 3, the revealed from the concentration dependencies $IC_{50}$ values for inhibiting the growth of EAC cells by some fractions, isolated from the three plant extracts, are presented.

## DISCUSSION

The results of investigations presented in this work manifested the toxicity of ethanol extracts of rose petals, pellicles of walnut kernels, melilot, leaves of sea buckthorn, sorrel and grape toward mice Ehrlich ascites carcinoma cells. Taking into account that bioactivity-directed fractionation must be carried out to identify compounds which can serve as candidates for preclinical testing, several constituents of these extracts were isolated and studied for cytotoxicity against both EAC cells and MBLs (Fig. 4). The observed higher cytotoxic efficacy of crude extracts compared with the cytotoxicity of the isolated components can be explained by synergy effect due to the probable presence of substances which enhance the activity of the components responsible for the researched toxicity (Gilbert and Alves, 2003).

The observed relatively mild toxicity of coumarins from three plants toward both EAC cells and MBLs (Fig. 4), despite the known cytotoxicity of such compounds (Luo *et al*., 2012; Riveiro *et al*., 2010) was in accordance with their low antioxidant activity (Table 2). Interestingly, among several coumarins, EAC cells most effectively were inhibited by Cm2 from RP with the absorbance spectrum (Fig. 3a, curve 2) characteristic for monomer coumarins (Kim *et al*., 2003). Cm from SL and Cm1 from RP with the absorbance spectra, characteristic for dimeric form (Fig. 3a, curve 1), provided relatively low inhibition of EAC cells, having practically no effect on MBLs (Fig. 4). The DNA-comet assay (Fig. 5) demonstrated pronounced genotoxicity of Cm1 from GL, characterizing the general mechanism of cytotoxicity of coumarins as preferentially apoptotic.

The therapeutic benefit of medical plants (particularly, the cytotoxicity and anticancer activity), is often attributed to their antioxidant properties. However, the obtained in our research cytotoxicity of six phenol glycosides from three plants (Fig. 4) did not correspond to their various antioxidant activities (Table 2). Contrary to high antioxidant activity, PhG2 from GL and PhG3 from RP possessed low cytotoxicity toward EAC cells (Table 3) and had no effect on MBLs. Whereas PhG from SL, PhG1 from GL, PhG1 and PhG2 from RP with relatively lower antioxidant activity possessed rather strong cytotoxicity toward EAC cells (Table 2 and 3), practically having no effect on the MBLs. This observation enhances the chemotherapeutic significance of phenol glycoside fractions from all the three studied edible plants: Rose petals, leaves of sorrel and grape. Phloroglucide fraction from GL demonstrated similar peculiarity. We presume that these compounds can be considered as candidates for preclinical testing of their anticancer properties. The work, directed to the elucidation of the molecular structure of the perspective compounds is underway.

The toxicity of the ethanol extracts from three plants toward mouse blood lymphocytes (Fig. 4) was in keeping with their antioxidant features (Table 2). However, the toxicity to the Ehrlich ascites carcinoma cells of the extract from sorrel leaves, despite its lower antioxidant activity, was

higher than the toxicity of the extracts both from rose petals and grape leaves with higher antioxidant activities. Unlike this peculiarity of sorrel leaves, the ability of ethanol extract of *Rumex* L. fruit to kill cancer cells, reported by Wegiera *et al.* (2012), was in accordance with the high antioxidant activity of methanol extract of ripe fruits of the plant, observed by Maksimovic *et al.* (2011). The anti-cancerous property of methanol extract of sorrel roots was also in compliance with its free radical scavenging activity (Shiwani *et al.*, 2012).

Let us note that, like the extract of sorrel leaves, the anthracene derivative from it also demonstrated relatively low toxicity toward normal cells and strong toxicity to EAC cells. All these observations-the strong toxicity to EAC cells of PhG, AD and the extract of SL as a whole, together with their low action on normal cells, underlines the preference of sorrel leaves as an anticancer remedy.

In general, based on the described results of the implemented investigation, one can conclude, that the extracts of rose petals, leaves of sorrel and grape, as well as some of their constituents can be recommended as sources for searching new anticancer medicaments.

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