https://doi.org/10.31925/farmacia.2022.2.9 **ORIGINAL ARTICLE**

PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITY OF ETHANOLIC EXTRACT OF *ROSA X DAMASCENA* MILL. CULTIVATED IN THE WESTERN REGION OF ROMANIA

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Abstract

Rosa x damascena Mill. has pharmacological properties that are partially attributed to their abundance in phenolic compounds. We investigated the alcoholic extract from the plants petals. Microscopic examination of *Rosa x damascena* Mill. petals showed that both of the adaxial and abaxial epidermal cells contained pigments. The antimicrobial activity of the extract was determined by the disc diffusion method. The most sensitive antimicrobial effect was on the strain *Pseudomonas aeruginosa*. The SOD-like activity of *Rosa damascena* alcoholic extract has been tested both *in vitro* and *in vivo*. Three cell lines were used for determining the cytotoxicity of *Rosa damascena* alcoholic extract, a human cervical carcinoma line (HeLa), a human radical growth phase melanoma cell line (WM35) and a normal fibroblastic epithelial cell line (HFL1) by MTT method. The *Rosa damascena* extract presents SOD-like activity and is cytotoxic on HeLa and WM35 cell lines. Due to these results, *Rosa x damascena* extracts can be used for their bioactive potential.

Rezumat

Rosa x damascena Mill. are proprietăți farmacologice care sunt parțial atribuite abundenței lor în compuși fenolici. Am investigat extractul alcoolic din petalele acestei plante. Examinarea microscopică a petalelor a arătat că ambele celule epidermice adaxiale și abaxiale conțin pigmenți. Activitatea antimicrobiană a extractului a fost determinată prin metoda de difuzie a discului. Cel mai sensibil efect antimicrobian a fost asupra tulpinii *Pseudomonas aeruginosa*. Activitatea SOD a extractului alcoolic din *Rosa damascena* a fost testată atât *in vitro*, cât și *in vivo*. Au fost utilizate trei linii celulare pentru determinarea citotoxicității extractului alcoolic, o linie de carcinom cervical uman (HeLa), o linie celulară de melanom în fază de creștere radicală umană (WM35) și o linie celulară epitelială fibroblastică normală (HFL1) prin metoda MTT. Extractul de *Rosa damascena* prezintă activitate asemănătoare SOD și este citotoxic pe liniile celulare HeLa și WM35. Datorită acestor rezultate, extractele de *Rosa x damascena* pot fi utilizate pentru potențialul lor bioactiv.

Keywords: bioactive compounds, antioxidant capacity, antimicrobial activity, HPLC method, diffusion method, SOD-like activity, cytotoxicity

Introduction

Rosa x damascena Mill. (*Rosaceae*), is an ornamental plant of the genus Rosa with fragrant effect and several reported pharmacological properties [13, 14]. Is a rose hybrid, derived from *Rosa moschata*, *Rosa gallica* and *Rosa fedtschenkoana*.

Studies have been carried out on flowers, petals, hips and leaves of *Rosa x damascena* Mill. (*Rosaceae*) and terpenes, glycosides, flavones, anthocyanins, myrcene,

vitamin C, carboxylic acids, quercetin and kaempferol were identified. Oils, organic acids, tannins and geraniol also have been identified in flowers. Studies showed that one of the major components of oils was geraniol. *Rosa x damascena* Mill. (*Rosaceae*) has pharmacological effects on the central nervous system. Effects on the respiratory system, cardiovascular, laxative, antidiabetic, antimicrobial, anti-HIV, anti-inflammatory and anti-oxidant effects, antitumour and cytotoxic effects against various cancer cells are other effects of this plant [22, 35, 60]. They act through different mechanisms, but one of the most important is the induction of apoptosis in cancer cells followed by a favourable prognosis [24, 61].

The objectives of this study are to present the microscopic examination, chemical composition, the total content of polyphenols, flavones and anthocyanins and the presentation of the antioxidant capacity, anti-microbial, SOD-like, antitumour activity and HPLC analysis of the alcoholic extract of *Rosa x damascena* Mill. (*Rosaceae*) collected from a region of Bihor County, Romania. Our scientific interest is that the studied medicinal plants, after phytochemical characterization and evaluation of pharmacological properties to be included in a useful collection for both specialists in pharmaceutical technology and of medical field.

Materials and Methods

Microscopic Examination of Rosa x damascena Mill. petals

Rosa x damascena Mill. petals were used in our study. The plant samples arose from unpolluted areas of the cultivated flora of Salonta (town located in North-Western part of Romania, in Bihor County) and were carefully collected and selected in May 2019. A specimen of the species is kept in the Herbarium of Pharmacy Department, Faculty of Medicine and Pharmacy, University of Oradea, Romania, code: UOP 05225.

The study was conducted in accordance with the guiding principles of European Community and the University of Oradea, Romania, Faculty of Medicine and Pharmacy, Ethics Committee.

From the collected petals *of Rosa x damascena* Mill. samples were made microscopical sections. The microscopic analysis was conducted using the OPTIKA B-383PL light microscope, equipped with Proview digital camera and software. Cross sections were made at the level of freshly petals. The obtained cross sections were analysed using the objective 10X. After capturing and saving the images with Proview digital camera and soft-ware, the sections have not been preserved.

Reagents

All chemicals and reagents used in this study have a high degree of purity. Sodium carbonate and gallic acid were provided by Fluka, Switzerland; all the other reagents used were from Sigma Aldrich, Germany. Twice-distilled water was obtained using a Milli-Q system (Millipore, Bedford, MA, USA).

Preparation of the extract

The petals were dried at 40°C, for 120 minutes using an UTD-1295 Laboratory oven 50 lt. The preparation method used to obtain the alcoholic extract solution was maceration, method of extraction with alcohol at a temperature of 20°C. Over 10 g petals of *Rosa x damascena* Mill. 100 mL of 70% ethanol was added and left at 20°C, for 7 days. The residue was removed by decantation and the solvent was removed using a Hei-VAP rotary evaporator (Heidolph Instruments, Berlin, Germany). All subsequent determinations were performed in triplicate. The extraction yield for the analysed extract was 21%.

Total polyphenols content (TPC)

TPC was determined using Folin-Ciocâlteu method described in literature [59], a widely used colorimetric method, and the total polyphenols content was calculated as gallic acid equivalents/100 g dry vegetable product (mg equivalent of gallic acid (GAE)/100 g dry weight (DW)).

Total flavonoid content (TFC)

TFC was performed by the colorimetric method, using $AlCl_3$, which forms a complex with the carbonyl groups of flavones. Results were expressed as mg quercetin equivalent (QE) *per* 100 g DW, using a calibration curve [36].

Total anthocyanin content (TAC)

TAC was determined using a method described in the literature [19]. In this method, the samples were diluted (5:95, v/v) in 1% HCl in methanol to obtain an absorbtion of 0.200 - 1.000 at 530 nm, and the results were expressed in mg cyanidin/100 g DW.

Analysis of phenolic compounds by HPLC

A new LC-MS method described in literature [54] was used to identify 10 polyphenols in *Rosa x damascena* Mill. extract using as standards: protocatechuic, p-coumaric and gallic acid, kaempferol, kaempferol-3-*O*-rhamnoside, hyperoside (quercetin-3-*O*-galactoside), iso-quercitrin (quercetin-3-*O*-glucoside), rutin (quercetin-3-*O*-rutinoside), quercitrin (quercetin-3-*L*-rhamnoside) and quercetin (quercitin).

The chromatographic separation was performed on an analytical column (Zorbax SB-C18, 100 mm x 3.0 mm i.d., 3.5 µm). The qualitative detection of the compounds was performed on MS mode (SIM-MS). The MS system operated using an electrospray ion source in negative mode (capillary +3000 V, nebulizer 60 psi (nitrogen), dry gas nitrogen at 12 L/min, dry gas temperature 360ºC). The quantification of a compound previously detected in MS mode was made in UV at 330 nm for the polyphenolic acids until 17 minutes and 370 nm for the detection of flavonoids and their aglycones until the end of analysis.

Determination of antioxidant capacity

DPPH method. The method uses as reagent 1,1-diphenyl-2-picril-hydrazil, an intense purple radical

reagent that turns pale yellow or colourless when neutralized by flavones in plant extracts. The anti-oxidant activity was calculated as the percentage of inhibition [36].

FRAP method. This method is based on the reduction reaction of the ferric tripyridyltriazine complex (Fe(III)-TPTZ) to the ferrous tripyridyltriazine complex (Fe(II)-TPTZ). The stock solutions included: 300 mM acetate buffer; 270 mg $FeCl_3$ x 6 H_2O dissolved in 50 mL distillated water; 150 mg TPTZ and 150 µL HCl, dissolved in 50 mL distillated water. The working FRAP solution was freshly prepared by mixing 50 mL acetate buffer, 5 mL $FeCl_3$ x 6 H_2O solution and 5 mL TPTZ solution. The results were expressed as µmol Trolox/100 mL [52].

CUPRAC method. The principle of this method is based on the reduction of the cupric ion (Cu^{2+}) to the cuprous ion (Cu^{+}) 0.25 mL $CuCl_2$, 0.25 mL ethanolic neo-cupreine solution, 0.25 mL acetate buffer, rose alcoholic extract and double distilled water were used, left in the dark at room temperature for 0.5 hours and the absorbance was read at 450 nm from the blank. The results were expressed as µmol Trolox/mL rose extract according to the calibration curve [37].

ABTS method. The method is based on the ability of antioxidants to reduce cation radical ($ABTS^{+}$), a green-blue chromophore that absorbs at 734 nm, the method being compared to the Trolox standard. $ABTS^{+}$ is produced by reacting stock solution ABTS (7 mM) with potassium persulfate (2.45 mM) for 12 - 16 h. In order to study the antioxidant activity, $ABTS^{+}$ solution is diluted with ethanol until an absorbance of 0.70 ± 0.02 to 734 nm is obtained. The results were expressed in µmol Trolox/mL extract [6, 64].

Antimicrobial activity

The antimicrobial activity of the alcoholic extract (from the petals of *Rosa x damascena* Mill. flowers) was performed by *in vitro* testing, using the Kirby-Bauer diffusion method [18]. Four reference strains were used: *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus* ATCC 29212 and four wild species: *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus* and *Staphylococcus aureus* that were isolated from clinical human cases.

The Mueller-Hinton Agar culture medium (Biomaxima, Poland) was inoculated with normalized bacterial inoculum (0.5 McFarland units). As a positive control, standard Amoxiclav discs (20/10 µg) were used, and as negative controls, paper discs soaked in distilled water (20 µL). The samples were stored at 37°C for 18 hours, then the diameters of the inhibition zones were measured and the arithmetic mean for each extract was calculated.

SOD-like activity

In vitro SOD-like activity. Xanthine (1.5 x 10^{-4} M) and xanthine oxidase in 50 mM potassium phosphate buffer, pH = 7.8 were used to generate a reproductible and constant flux of superoxide anions, that were detected by the reduction of nitroblue tetrazolium (NBT) (5.6 x 10^{-5} M) to blue formazane, which was quantified spectrophotometrically at 560 nm. To determine the SOD activity of the plant alcoholic extract, the percentage inhibition of NBT reduction was used. The IC_{50} values were determined from plots of % inhibition *versus* plant alcoholic extract concentration [30, 51]. All reagents for these assays were obtained from Sigma, USA, and the determinations were performed with a SPECORD 200 PLUS Spectro-fotometer, Germany.

In vivo SOD-like activity. The SOD-like activity of *Rosa x damascena* alcoholic extract was evaluated using a strain of *Saccharomyces cerevisiae* Δsod1 (ATCC96687), which has the ability to delete/insert the SOD_1 gene encoding the synthesis of Cu_2Zn_2SOD. Yeast cells were grown in YPD reach medium (1% yeast extract, 2% peptone and 2% glycerol). Solid media contained 1.5% agar. Cell suspensions were poured into Petri dishes and allowed to solidify at room temperature. Paper disks measuring 6 mm in diameter containing 5 µL of a 5 mM menadione solution in ethanol or 5 µL of 17.5% H_2O_2 have been used. The diameters of clear zones around the disks, measured after 3 days of incubation at 28°C, where taken as a quantitative estimate of the protective action [43].

Cell culture and Cytotoxicity assays

Cell culture. Three cell lines were used for determining the cytotoxicity of *Rosa damascena* alcoholic extract, a human cervical carcinoma line (HeLa), a human radical growth phase melanoma cell line (WM35) and a normal fibroblastic epithelial cell line (HFL1). The cell lines were maintained in DMEM (Sigma-Aldrich, USA), supplemented with 10% foetal bovine serum (FCS, Hyclone), 1 mM glutamine (Sigma-Aldrich, USA), 1% antimycotic antibiotic 100x (Sigma-Aldrich, USA). The cells were cultured at 37°C in an atmosphere of 5% CO_2 and 95% relative humidity [32].

Cytotoxicity assays. Cytotoxicity studies on tumoural cell lines was performed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma Aldrich, USA). For all three cell lines, the cells were plated (1 × 10^5 cells/well) in 96-well plates for 24 h in normal propagation media. The positive control was represented by Cisplatin (Ebewe Pharma, Austria) in the same concentration as the ethanolic extract. The negative controls were represented by cell lines cultivated in normal expansion medium (untreated cells) and the internal control by 70% v/v ethanol. The cells viability was determined using the MTT assay at 24, 48 and respectively 72 h. The formazan particles were solubilized with dimethyl sulfoxide (DMSO) (Sigma) and the absorbance of each sample was read at 550 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). Each experiment was carried out in triplicate [42, 62].

Statistical analysis

For all data that are presented as the mean ± SD, Gaussian distribution was checked by Shapiro - Wilk normality test, test two-way ANOVA followed by Bonferroni post-test was done for pair-wise comparisons. Statistical significance was set at $p < 0.05$ (95% confidence interval). Values, including IC_{50} were generated using GraphPad Prism version 5.0 for Windows, GraphPad Software, San Diego California USA.

Results and Discussion

Microscopic Examination of Rosa x damascena Mill. *petals*

The mature petals of the corolla were selected for microscopic observations. The cross sections were cut from the fresh petals, with special attention to the structure of the epidermal cells (Figure 1).

The adaxial epidermis formed a single cell layer (1ad). We found conical papillae in the adaxial epidermis. The walls of the papilla cells were thin, covered by a layer of cuticle. The mesophyll was made up of several layers of parenchymal cells (1m). The collateral vascular bundles were located in the middle of the petal mesophyll (1vb).

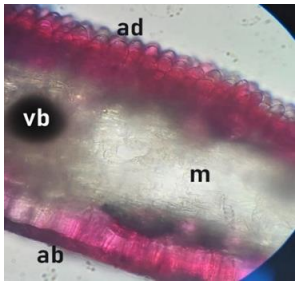

Figure 1.
Cross section of petals of *Rosa x damascena* Mill. (10x)
ad – adaxial epidermis; ab – abaxial epidermis;
m – mesophyll; vb – vascular bundle

The abaxial epidermis formed a single cell layer and was composed of cells with wavy anticlinal walls (1ab). Both of the adaxial and abaxial epidermal cells contained pigments, as can be seen in Figure 1.

Determination of the content of polyphenols, anthocyanins and flavones in the alcoholic extract of Rosa x damascena Mill.

The total anthocyanins content (782.16 ± 0.05 mg Cy/100 g DW**)** is the highest value of compounds found in the alcoholic extract, followed by the total polyphenolic content (321 ± 0.03 mg GAE/100 g DW) and total flavones (32.4 ± 0.01 mg QE/100 g DW).

HPLC analyses

Qualitative characterization of the samples was performed by HPLC analysis. Using the standards mentioned at "Analysis of phenolic compounds by HPLC" section, they were identified the compounds presented below. The aglycones have the same therapeutic profile as quercetin but with superior bio-availability. In our study, quercetin-3-O-β-D-gluco-pyranoside (isoquercitrin) was the most abundant phenolic compound (594.127 µg/mL), followed by hyperozid (quercetin-3-*O*-β-D-galactoside) (235.918 µg/mL), quercitrin (quercetrin-3-*O*-α-L-rhamnoside) (153.484 µg/mL), rutozid (quercetin-3-*O*-β-D-rutinoside) (64.542 µg/mL) and quercetin (53.193 µg/mL) while low amounts of kaempferol-3-rhamnoside (38.879 µg/mL), kaempferol (18.922 µg/mL) and p-cumaric acid (1.464 µg/mL), gallic acid (7.030 µg/mL) and protocatechuic acid (2.089 µg/mL) were detected in our samples.

Antioxidant capacity

Antioxidant capacity was measured using DPPH, CUPRAC, FRAP and ABTS assay. In the analysis of antioxidant activity, a 10 µmol/mL gallic acid solution was used as a control sample. For all antioxidant assays there were obtained high values: DPPH (94.11 ± 0.01%), CUPRAC (555.316 ± 0.06 µmol Trolox/mL), FRAP (354.43 ± 0.03 µmol Trolox/mL) and ABTS (76.351 ± 0.02 µmol Trolox/mL).

Antimicrobial activity

The results of the antimicrobial activity are presented in the Table I.

Table I
Results of antimicrobial activity

Samples	**Conc.**	***S. aureus* ATCC 25923**	***E. coli* ATCC 25922**	***P. aeruginosa* ATCC 27853**	***Enterococcus* ATCC 29212**	***P. aeruginosa***	***E. coli***	***S. aureus***	***Enterococcus***
		Zone of growth inhibition (in mm diameter)							
Alcoholic extract *Rosa x damascena*	100 mg/mL	15	17	**25**	21	**25**	10	11	15
Amoxicillin/ clavulanic acid	20/10 µg	35	28	28	27	25	27	35	21
Cefuroxime	30 µg	35	25	not tested	not tested	not tested	28	31	not tested
Cefixime	5 µg	not tested	27	25	not tested	25	25	not tested	not tested
Cefaclor	30 µg	28	25	not tested	not tested	not tested	27	27	not tested
Ofloxacin	5 µg	27	31	20	not tested	21	28	27	not tested
Clarithromycin	15 µg	29	not tested	not tested	not tested	not tested	not tested	28	not tested
Erythromycin	15 µg	29	not tested	not tested	25	not tested	not tested	26	24

Samples	Conc.	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*Enterococcus* ATCC 29212	*P. aeruginosa*	*E. coli*	*S. aureus*	*Enterococcus*
		Zone of growth inhibition (in mm diameter)							
Azithromycin	15 µg	27	not tested	not tested	21	not tested	not tested	21	21
Distilled water		6	6	6	6	6	6	6	6

Testing of *Rosa x damascena* extract by evaluating the diameter of the inhibition zone on *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 25922 and *Enterococcus* ATCC 29212 concluded that the anti-bacterial effect on the tested species was moderate. However, the greatest antimicrobial effect was on *Pseudomonas aeruginosa* ATCC 27853, comparable to some antibiotics tested (Amoxiclav®, cefixime, ofloxacin). From the four wild strains isolated from human clinical cases, in terms of the antibacterial effect of the plant extract, the largest effect was on the strain *Pseudomonas aeruginosa*, while on *E. coli* and *Staphylococcus aureus* it had a weak effect. On the clinical strain isolated from *Enterococcus*, *Rosa x damascena* Mill. extract had a moderate antibacterial effect.

SOD-like activity

In vitro SOD-like activity. The SOD-like activity of *Rosa damascena* alcoholic extract was tested by an indirect method using the xanthine/xanthine oxidase system as the source of superoxide radicals. The IC_{50} value was found 0.159 µg/mL. IC_{50} value for native Cu_2Zn_2SOD is 0.02 µg/mL.

In vivo SOD-like activity. SOD-like *in vivo* activity of *Rosa damascena* alcoholic extract was quantified through a method based on the protection against free radicals provided by the extract to the yeast *Saccharomyces cerevisiae* [44]. The diameter of the inhibition area for *Rosa damascena* alcoholic extract at 10 µg/mL, using menadione and H_2O_2 was 4.9 ± 0.08 respectively 5.1 ± 0.03 cm. At a concentration of 30 µg/mL using menadione and H_2O_2 was 4.7 ± 0.12 and 5.1 ± 0.08 cm. The diameter of inhibition area for the plant alcoholic extract at a concentration of 50 µg/mL was 4.6 ± 0.09, respectively 5 ± 0.13 cm.

Cell culture and Cytotoxicity assays

Three cell lines were used for determining the cyto-toxicity of *Rosa damascena* alcoholic extract, a human cervical carcinoma line (HeLa), a human radical growth phase melanoma cell line (WM35) and a normal fibroblastic epithelial cell line (HFL1) by MTT method. The results of the cytotoxicity assays are presented in Table II. As positive control we used Cisplatin.

Table II

The results of the cytotoxicity assays

Cells	Compound	IC_{50} (µg/mL)		
		24 h	**48 h**	**72 h**
WM35 cells	*Rosa damascena*	41.19 ± 0.37	39.08 ± 0.08	22.98 ± 0.06
	Cisplatin	18.04 ± 0.05	13.97 ± 0.11	9.60 ± 0.08
HeLa cells	*Rosa damascena*	29.00 ± 0.11***	20.87 ± 0.02***	12.39 ± 0.35***
	Cisplatin	10.03 ± 0.19	5.36 ± 0.05	0.74 ± 0.02
HFL1 cells	*Rosa damascena*	55.21 ± 0.34***	47.28 ± 0.02***	32.42 ± 0.45***
	Cisplatin	11.98 ± 1.01###	7.87 ± 0.12###	1.25 ± 0.17###

*** $p < 0.001$ compared to *R. damascena* – WM35 treated cells; ### $p < 0.001$, compared to *R. damascena* – HFL1 cells

The composition variability of medicinal plants is influenced by a number of factors (environmental, climatic conditions, etc.) which led us to evaluate the pharmacological activity of bio-compounds extracted from the cultivated *Rosa species* and in the geographical area of Romania [53]. These small differences can be explained by the variation of the composition of the plants depending on the geographical area and the technique of preparation of the extract. There is no standardized protocol for determining antimicrobial and antineoplastic activity; the results are influenced by the chosen method [54].

Our results are different from the results reported by other authors, but they studied hydro-alcoholic extracts of *Rosa x damascena* Mill. (TPC = 217.728 ± 0.13 mg GAE/g and a TFC = 22.8 ± 0.18 mg CE/g) [65]. In the case of fresh flower extract, it was reported a TPC = 276.02 ± 2.93 mg GAE/g, also for spent flower extract, TPC = 248.97 ± 2.96 mg GAE/g [60]. Total phenolic content of dry rose petal extracts expressed as mg GAE/100 g dry extract ranged between 781 - 2006 [26].

Five different rose hip species [23] collected from Turkey were studied noticed high values for TPC from *R. gallica* (3151 mg GAE/100 g DW) and *Rosa canina* (3108 mg GAE/100 g DW). Also, in their study they concluded that total phenolic contents found in the samples were significantly influenced by the species even if total flavonoid content was similar in all the examined species. Regarding the content of flavones in literature other authors compared the flavonoid content of eleven *Rosa sp*. and the values obtained were between 20 - 98 mg QE/100 g DM (dried matter of freeze-dried hips) [50, 63].

Two of the major glycosidic forms of the natural flavonol quercetin are isoquercitrin and rutin [16, 20]. Isoquercitrin, the most abundant phenolic compound from our samples is one of the glucosides of quercetin. Studies showed that isoquercitrin inhibits the formation of advanced glycation end-products (AGEs) that is one of primary pathologic mechanisms implicated in diabetic complications [38]. Also, quercetin and rutin inhibit the generation of inflammatory mediators (leukotriene LTB4 and prostaglandin E2) in human neutrophils [15]. Flavones have demonstrated protection of the brain due to their ability to modulate intracellular signals, promoting cellular survival [21]. Cellular antioxidant and inflammatory activities of quercetin, isoquercitrin, hyperoside and quercitrin depend on the presence and type of monosaccharide attached to the compound [17]. From *Rosa x damascena* methanolic extracts and ethyl acetate fraction was identified 7.75 mg/g, and 65.27 mg/g quercetin, while gallic acid was reported as trace in methanolic extract and 10.01 mg/g in ethyl acetate fraction [1]. In the methanolic extract of fresh and spent rose flower was detected quercetin (0.14 - 0.40 mg/g) and syringic acid (0.04 - 0.54 mg/g), also low amounts of gallic acid that ranged from 1.91 to 28.18 mg/g [10].

In samples prepared from *R. damascena* Mill. distilled petals were reported 0.189 mg QE/g [56]. In fact, *in vitro* studies have ascertained that quercetin like other flavones may strongly inhibit the production of both nitric oxide and tumour necrosis factor by Kupffer cells when stimulated by injury, quercetin also protects cells against injury caused by X-rays, and may act in preventing carcinogenesis [8].

Protocatechuic acid is a type of widely distributed naturally occurring phenolic acid and has structural similarity with gallic acid, caffeic acid, vanillic acid and syringic acid which are well-known antioxidant compounds. It seems to have chemopreventive potential because it inhibits the *in vitro* chemical carcinogenesis and exerts proapoptotic and antiproliferative effects in different tissues. The mechanism of the chemopreventive action of protocatechuic acid is mostly associated with antioxidant activity, including inhibition of generation as well as scavenging of free radicals and upregulating antioxidant enzymes.

Our findings are in accordance with most of the studies, related to the antioxidant activity and the structure of the chemical compound [12]. The antioxidant activity of phenolic compound is due to their ability to scavenge free radicals, donate hydrogen, atoms or electron, or chelate metal cations [9]. Using some *Rosa species* was analysed antioxidant capacity of rosehips and were obtained FRAP values that ranged from 228.2 - 464.8 mM ascorbic acid/g DW [39] and were similar to those found by other researchers [24-29]. Beside FRAP assay, researcher used CUPRAC method to characterize antioxidant properties of *Rosa caninae flos* and rosehip fruits [26]. DPPH and ABTS methods were also used to study antioxidant properties of some *Rosa sp*.

The extracts from plant products are tested antimicrobial for information on safe and effective use [41].

The antimicrobial effect could be attributed to the polyphenol composition of the extract [10, 14]. Transmission electron microscopy determined the morphological changes of the bacteria treated with *Rosa x damascena* extract, which indicates that the polyphenols in the extract have as primary inhibitory action, damage to bacterial cell membranes [33, 66]. In literature were reported studies that show the mechanism of actions against Gram-negative bacteria that implies the inhibition of adenosine triphosphate generation and membrane disruption. Bacterial membranes contain a number of differing enzymes with ATPase activity including, ATP dependent transport proteins and the F1F0 ATPase which is involved in ATP generation and cellular pH regulation [53]. Though inhibition of these functions would impair cell survival, that significant ATPase inhibition occurs at concentrations within the same range required for membrane disruption (5 to 10 mM) suggests that this is a secondary rather than a primary cause of cell death [28].

Regarding the mechanism of action against Gram-positive bacteria, due to the composition of outer membrane, active substances from plant extracts can alter not only such structures, but penetrate within the cell, leading to the denaturation of proteins and enzymes, the "unbalance" of the K^+ and H^+ ion concentration, until the modification of the entire cell morphology, which can lead to the death of the microorganism [45].

In a study [49] was demonstrated that the rose water has an antimicrobial effect on Candida albicans and methicillin-resistant *Staphylococcus aureus* (MRSA).

Our results are similar to those obtained in other studies reported in the scientific literature [3, 4, 46]. In a study published in literature [29] was studied the antimicrobial activity against 10 pathogenic microorganisms using ethanolic and aqueous extracts of *R. damascena* and the most antimicrobial effect was obtained for ethanolic extracts against *P. aeruginosa* ATCC 27853 at minimum inhibition concentration and minimum bactericidal concentration.

The SOD-like activity of *Rosa damascena* alcoholic extract was tested *in vitro* by an indirect method using the xanthine/xanthine oxidase system as the source of superoxide radicals and *in vivo* using a method based on the protection against free radicals provided by the plant alcoholic extract to the yeast *Saccharomyces cerevisiae*.

In our study, for *in vitro* SOD-like activity we found the IC_{50} value of *Rosa damascena* alcoholic extract superior to the IC_{50} value of native Cu_2Zn_2SOD (0.159 μg/mL > 0.02 μg/mL) [31]. We can conclude

that the extract has SOD mimetic activity, but it's inferior to native SOD.

The *in vivo* SOD-like activity of *Rosa damascena* alcoholic extract was determined using *Saccharomyces cerevisiae* strain. The SOD-mimetic activity of the plant extract on cell growth with a Δsod1 mutant treated with menadione or H_2O_2 had been evaluated [43]. The oxidative stress is produced by two oxidative agents: menadione which toxicity is due to the super-oxide radical production which toxicity is registered due to OH• radicals.

It will be considered that *Rosa damascena* alcoholic extract has a SOD-like activity if a decrease of the diameter of the inhibition zone is registered *versus* the control zone. The efficacy will then be evaluated by comparison of the diameter of the inhibition area for the plant extract and control.

In the presence of *Rosa damascena* alcoholic extract at 10, 30 and 50 μg/mL a significant reduction of the inhibition area was observed when the oxidative stress is produced by both menadione and H_2O_2.

The diameter of the inhibition area for *Rosa damascena* alcoholic extract in different concentrations, using menadione and H_2O_2 were determined. The reduction of the inhibition area was between 39 - 43% for the plant alcoholic extract against oxidative stress generated by menadione and between 32 - 33% against the oxidative stress generated by H_2O_2.

The protective activity of the plant extract did not seem to be dependent on extract concentration and the protection of the extract against free radicals generated by H_2O_2 is lower than in the case of free radicals generated by menadione.

The results suggest that *Rosa damascena* alcoholic extract was able to protect efficiently against super-oxide anions and it could be considered as a promising effective agent against toxicity of superoxide anion, improving significantly the growth of *Δsod1* strain. It supplies the Cu_2Zn_2SOD deficiency of the mutant. Due to this it is a potential therapeutic agent in the prevention and treatment of diseases mediated by free radicals.

Concerning the cytotoxic activity of *Rosa damascena* alcoholic extract, it would be beneficial the use of a natural extract in adjuvant cancer therapy.

Melanoma is a tumour of great significance because it has increased alarmingly among the white population in the last 50 years. It is the deadliest form of skin cancer: although melanoma represents less than 10% of all skin cancers, it is responsible for more than 75% of skin cancer-related death [47, 55, 57].

To investigate the *in vitro* cytotoxicity of *Rosa damascena*, the ethanolic extract was tested on two human cancer cell lines, WM35 and HeLa, while a normal fibroblastic epithelial cell line (HFL1) was used to test the selective cytotoxic potency. Cisplatin was used as positive control. A non-linear regression analysis of the dose-response curve determined half maximal inhibitory concentration (IC_{50}) values for cytotoxic activity and the results are presented in Table II. The results indicated a promising profile; *Rosa damascena* ethanolic extract had express a relevant *in vitro* cytotoxic activity and differences among the tested cancer cell lines and the normal fibroblastic epithelial one.

Rosa damascena ethanolic extract presented a better cytotoxic activity on HeLa cells then on WM35 cell line; the most intense *in vitro* cytotoxicity of *Rosa damascena* extract for HeLa cell line was at 72 h. The viability of both WM35 and HeLa cells was significantly decreased compared to the negative control (untreated cells) and to the internal control (70% v/v ethanol) for all concentrations. Cisplatin was more cytotoxic on HeLa cells than on WM35 cells and more cytotoxic than *Rosa damascena* ethanolic extract on both tumour cell lines.

On normal fibroblast (HFL-1 line), *Rosa damascena* ethanolic extracts showed a lower toxicity than the toxicity found on tumour cells, and it was significantly less toxic than Cisplatin, while Cisplatin inhibited indiscriminately both normal and tumour cells. The best difference was observed at 72 h, when IC_{50} values for HFL1 cells were 32.42 ± 0.45 for *Rosa damascena* extract and about only 1.25 ± 0.17 for Cisplatin ($p < 0.001$).

Thus, our results demonstrate the *in vitro* cytotoxic potency of *Rosa damascena* ethanolic extract on two tumour cell lines. HeLa cell line proved to be more susceptible than WM35 cells; varying *in vitro* and *in vivo* sensitivity between differing tumour cell types is documented in literature [7].

There are not many studies that reveal that *Rosa damascena* extracts and oils induced cytotoxic effects against various cancer cell lines. Some reports showed cytotoxic activities of *Rosa damascena* against human prostate, lung, liver and breast cancer cell lines [47, 68].

Some studies explored the mechanism of action of rose. Geraniol is one of the main components of rose which has been reported to work through various mechanisms such as induction of apoptosis as it increases the expression of apoptotic protein Bak and arrests the Go/G1 phase of cell cycle. It also reduces cdk2 activity and inhibits the 3-hydroxy-3-methyl-glutaryl-CoA reductase and ornithine decarboxylase activity. All these activities combined lead to the death of the cancer cells [40]. But, unfortunately, detailed mechanistic studies have not been conducted yet [47].

Many studies presented the prominent cytotoxic effects of methanolic extracts and rose oils of *R. damascena* Mill. cultivated in various countries against cancer cell lines, but this is the first comprehensive study made on ethanolic extract obtained from *Rosa x damascena* reared in Romania.

Correlating the results obtained in the phytochemical analysis, in the antioxidant and SOD-mimetic assays and in the citotoxicity assays,

the link between them becomes obvious and it can be concluded that the antiproliferative activity of *Rosa damascena* ethanolic extract may be due to an antioxidant mechanism. Some reports also support the relationship of cytotoxicity with antioxidant activity [34].

The high total polyphenols, flavones and anthocyanins content revealed that rose petals reared up in Romania represent a promising source of phenolic compounds which might be used as functional food ingredients and might be implicated in different antioxidant activity and therapeutic applications of this plant.

We can say that the extract of *Rosa x damascena* Mill. investigated in this paper possess a good anti-bacterial effect on *Pseudomonas aeruginosa* strain and a week to moderate antibacterial effect on Gram-positive and Gram-negative bacteria. Due to these results, *Rosa x damascena* extracts can be used in products with antiseptic action for eye washing in patients with conjunctivitis, dry eye; in patients with folliculitis, psoriasis; in treatment of aphthous stomatitis or other disorders. *Rosa damascena* extract presents SOD-like activity, is cytotoxic on HeLa and WM35 cell lines and in non-cytotoxic on HFL1, which means that presents exhibiting selective cytotoxicity.

Although all *in vitro* experiments hold limitations with regards to possible *in vivo* efficacy, the results of this study are promising with regards to possible antioxidant, antimicrobian and adjuvant antineoplastic in cancer therapy and form a sound basis for future research. In this study, *Rosa x damascena* hydro-alcoholic extract was analysed extensively compared to other studies reported in scientific literature, also cytotoxicity assays and phytochemical results showed that hydroalcoholic extract can be used safely to develop new formulas with topical or oral use.

Conclusions

The results provided in the present study support this view that *Rosa x damascena* Mill. harvested in Romania have a wide range of natural source for the discovery of natural pharmaceuticals and can be used as prophylactic agents in the pathologic process of various disorders. Moreover, detailed chemical studies for the purification and identification of the bioactive compounds followed by pharmacological investigations and toxicological evaluation are needed to examine the mechanisms of action of these agents. Phytochemical screening and biological activity should be used together to completely identify the promising activity of the extracts or compounds derived from them.

Conflict of interest

The authors declare no conflict of interest.

References

1. Abdel Hameed ESS, Bazaid SA, Salman MS, Characterization of the Phytochemical constituents of *Taif Rose* and its antioxidant and anticancer activities. *Br J Pharm Res.*, 2013; 2013: 345465: 1-13.
2. Adamczak A, Buchwald W, Zielinski J, Mielcarek S, Flavonoid and organic acid content in rose hips (*Rosa L.*, sect. *Caninae* DC. EM. Christ.). *Acta Biol Cracov Bot.*, 2012, 54(1): 105-112.
3. Adwan G, Mhanna M, Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. *Middle-East J Sci Res.*, 2008; 3(3): 134-139.
4. Andoğan BC, Baydar H, Kaya S, Demirci M, Özbaşar D, Mumcu E, Antimicrobial activity and chemical composition of some essential oils. *Arch Pharm Res.*, 2008; 25(6): 860-864.
5. Androutsopoulou C, Christopoulou SD, Hahalis P, Kotsalou C, Lamari FN, Vantarakis A, Evaluation of Essential Oils and Extracts of *Rose Geranium* and Rose Petals as Natural Preservatives in Terms of Toxicity, Antimicrobial, and Antiviral Activity. *Pathogens*, 2021; 10(4): 494: 1-16.
6. Arnao MB, Cano A, Alcolea JF, Acosta M, Estimation of free radical-quenching activity of leaf pigment extracts. *Phytochem Anal.*, 2001; 12(2): 138-143.
7. Aung TN, Qu Z, Kortschak RD, Adelson DL, Understanding the effectiveness of natural compound mixtures in cancer through their molecular mode of action. *Int J Mol Sci.*, 2017; 18(3): 656: 1-20.
8. Baghel SS, Shrirastava N, Baghel RS, Agrawal P, Rajput S, A review of quercetin: Antioxidant and anticancer properties. *World J Pharm Pharmaceut Sci.*, 2012; 1(1): 146-160.
9. Balasundram N, Sundram K, Saman S, Phenolic compound in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.*, 2006; 99(1): 191-203.
10. Baydar H, Sagdiç O, Özkan G, Karadogan T, Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Control*, 2003; 15(3): 169-172.
11. Baydar NG, Baydar H, Phenolic compounds, antiradical activity and antioxidant capacity of oil-bearing rose (*Rosa damascena* Mill.) extracts. *Ind Crop Prod.*, 2013; 41: 375-380.
12. Bidian C, Mitrea DR, Tatomir C, Perde-Schrepler M, Lazar C, Chetan I, Bolfa P, David L, Clichici S, Filip GA, Muresan M, Micle O, *Vitis vinifera* L. and *Sambucus nigra* L. extracts attenuate oxidative stress and inflammation in femoral ischemia. *Farmacia*, 2021; 69(1): 59-67.
13. Sezer Senol F, Orhan IE, Kurkcuoglu M, Hassan Khan MT, Altintas A, Sener B, Baser KHC, A mechanistic investigation on anticholinesterase and antioxidant effects of rose (*Rosa damascena* Mill.). *Food Res Int.*, 2013; 53(1): 502-509.
14. Boskabady HM, Shafei NM, Saberi Z, Amini S, Pharmacological Effects of *Rosa damascena*. *Iran J Basic Med Sci.*, 2011; 14(4): 295-307.
15. Bouriche H, Miles FA, Selloum L, Calder PC, Effect of *Cleome arabica* leaf extract, rutin and quercetin

on soybean lipoxygenase activity and on generation of inflammatory eicosanoids by human neutrophils. *Prostaglandins Leukot Essent Fatty Acids*, 2005; 72(3): 195-201.
16. Chen Q, Li P, Xu Y, Li Y, Tang B, Isoquercitrin inhibits the progression of pancreatic cancer *in vivo* and *in vitro* by regulating opioid receptors and the mitogen-activated protein kinase signalling pathway. *Oncol Rep.*, 2015; 33(2): 840-848.
17. Choi SJ, Tai BH, Cuong NM, Kim YH, Jang HD, Antioxidative and anti-inflammatory effect of quercetin and its glycosides isolated from mampat (*Cratoxylum formosum*). *Food Sci Biotech.*, 2012; 21(2): 587-595.
18. Silva V, Igrejas G, Falco V, Santos TP, Torres C, Oliveira AMP, Pereira JE, Amaral JS, Poeta P, Chemical composition, antioxidant and antimicrobial activity of phenolic compounds extracted from wine industry by-products. *Food Control*, 2018; 92: 516-522.
19. Connor AM, Luby JJ, Tong CBS, Variability in antioxidant activity in blueberry and correlations among different antioxidant activity assays. *J Am Soc Hortic Sci.*, 2002; 127(2): 238-244.
20. Cushnie TPT, Lamb AJ, Recent advances in understanding the antibacterial of flavonoids. *Int J Antimicrob Agents*, 2011; 38(2): 99-107.
21. Dajas F, Rivera-Megret F, Blasina F, Arredondo F, Abin-Carriquiry JA, Costa G, Echeverry C, Lafon L, Heizen H, Ferreira M, Morquio A, Neuroprotection by flavonoids. *Braz J Med Biol Res.*, 2003; 36(12): 1613-1620.
22. Dar RA, Shahnawaz M, Qazi PH, General overview of medicinal plants: A review. *JPHYTO*, 2017; 6(6): 349-351.
23. Demir N, Yildiz O, Alpaslan M, Hayaloglu A, Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (*Rosa* L.) fruits in Turkey. *LWT Food Sci Technol.*, 2014; 57(1): 126-133.
24. Deliorman OD, Orhan N, Demir O, Konuklugil B, Phenolic content, antioxidant and *in vitro* antidiabetic effects of thirteen marine organisms from Mediterranean sea. *Farmacia*, 2021; 69(1): 68-74.
25. Gao X, Björk L, Trajkovski V, Uggla M, Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. *J Sci Food Agric.*, 2000; 80: 2021-2027.
26. Gavan A, Totoiu AM, Tamas M, Tomuta I, Dry rose petal extracts and compressed lozenges: Formulation and NIR – chemometric quantification of active principles. *Farmacia*, 2017; 65(4): 577-583.
27. Gavra DI, Pallag A, Marian E, Vicaș L, Jurca T, A comparative study of the amount of polyphenols, flavonoids and antioxidant capacity of *Rosae caninae flos versus Cynosbati fructus*. *AUO, Fascicle: Environ Protection*, 2018; 31: 23-30.
28. Gill AO, Holley RA, Inhibition of membrane bound ATPases of *Escherichia coli* and *Listeria monocytogenes* by plant oil aromatics. *Int J Food Microbiol.*, 2006; 111: 170-174.
29. Halawani EM, Antimicrobial activity of *Rosa damascena* petals extracts and chemical composition by gas chromatography-mass spectrometry (GC/MS) analysis. *Afr J Microbiol Res.*, 2014; 8(24): 2359-2367.
30. Hangan AC, Stan RL, Sevastre B, Gheorghe-Cetean S, Oprean L, DNA cleavage study and SOD-mimetic activity of a new Cu(II) complex. *Farmacia*, 2017; 65(3): 368-373.
31. Hangan AC, Stan RL, Turza A, Oprean LS, Pall E, Gheorghe-Cetean S, Sevastre B, Synthesis, crystal structures, characterization and antitumor activities of two copper(II) complexes of a sulfonamide ligand. *Transit Met Chem.*, 2017; 42(2): 153-164.
32. Hangan AC, Vicaș LG, Lucaciu Stan RL, Pall E, Oprean LS, Ionescu CML, Andrei S, Sevastre-Berghian AC, Marian E, Sevastre B, Synthesis, characterization and biological activity of two new Copper (II) complexes with N-sulfonamide ligand. *Rev Chim.*, 2019; 70(11): 4060-4067.
33. Hirulkar NB, Agrawal M, Antimicrobial activity of rose petals extract against some pathogenic bacteria. *Int J Pharm Biol Arch.*, 2010; 1: 478-484.
34. Hou J, Sun T, Hu J, Chen S, Cai X, Zou G, Chemical composition, cytotoxic and antioxidant activity of the leaf essential oil of *Photinia serrulata*. *Food Chem.*, 2007; 103(2): 355-358.
35. Ghitea TC, El-Kharoubi A, Ganea M, Bimbo-Szuhai E, Nemeth TS, Ciavoi G, Foghis M, Dobjanschi L, Pallag A, Micle O, The Antimicrobial Activity of *Origanum vulgare* L. Correlated with the Gastrointestinal Perturbation in Patients with Metabolic Syndrome. *Molecules*, 2021, 26(2): 283: 1-17.
36. Kumar S, Sandhir R, Ojha S, Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. *BMC Res Notes*, 2014; 7: 560: 1-9.
37. Karaman S, Tutem E, Baskan KS, Apak R, Comparison of total antioxidant capacity and phenolic composition of some apple juices with combined HPLC– CUPRAC assay. *Food Chem.*, 2010; 120(4): 1201-1209.
38. Kim HY, Moon BH, Lee HJ, Choi DH, Flavonoid glycosides from the leaves of *Eucommia ulmoides* O. with glycation inhibitory activity. *J Ethnopharmacol.*, 2004; 93(2-3): 227-230.
39. Koczka N, Stefanovits Bányai É, Ombódi A, Total polyphenol content and antioxidant capacity of rosehips of some *Rosa* Species. *Medicines*, 2018; 5(3): 84: 1-10.
40. Kritika-Seghal R, Singh M, Essentials to kill the cancer. *Canc Therapy Oncol Int J.*, 2017; 4(5): 1-4.
41. Kurhade BB, Vite MH, Nangude SL, Antibacterial activity of *Rosa x damascena* miller. *Int J Res Pharma Biomed Sci.*, 2011; 2: 1015-1020.
42. Marian E, Duțeanu N, Vicaș L, Rusu G, Jurca T, Mureșan M, Micle O, Hangan AC, Stan RL, Ionescu C, Sevastre B, Pall E, Synthesis, characterization of inclusion compounds of amygdalin with β-cyclodextrin and sod-like activity and cytotoxicity on hela tumor cells. *Arab J Chem.*, 2020; 13(8): 6828-6837.
43. Marian E, Vicaș LG, Jurca T, Mureșan M, Pallag A, Stan RL, Sevastre B, Diaconeasa Z, Ionescu CML, Hangan AC, *Salvia officinalis* L. and *Verbascum phlomoides* L. chemical, antimicrobial, antioxidant and antitumor investigations. *Rev Chim.*, 2018; 69(2): 365-370.
44. Sas K, Robotka H, Toldi J, Vecsei L, Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *J Neurol Sci.*, 2007; 257(1-2): 221-239.

45. Marrufo T, Nazzaro F, Mancini E, Fratianni F, Coppola R, De Martino L, Agostinho AB, De Feo V, Chemical composition and biological activity of the Essential oil from Leaves of *Moringa oleifera* Lam. Cultivated in Mozambique. *Molecules*, 2013; 18(9): 10989-11000.
46. Mohaddese M, *Rosa damascena* as holy ancient herb with novel applications. *J Tradit Complement Med.*, 2015; 6(1): 10-16.
47. Mujtaba SF, Masih AP, Alqasmi I, Alsulimani A, Khan FH, Haque S, Oxidative-Stress-Induced Cellular Toxicity and Glycoxidation of Biomolecules by Cosmetic Products under Sunlight Exposure. *Antioxidants (Basel)*, 2021; 10(7): 1008: 1-10.
48. Nađpal JD, Lesjak MM, Šibul FS, Anačkov GT, Četojević-Simin DD, Mimica-Dukić NM, Beara IN, Comparative study of biological activities and phyto-chemical composition of two rose hips and their preserves: *Rosa canina* L. and *Rosa arvensis* Huds. *Food Chem.*, 2016; 192: 907-914.
49. Naho Maruyama Shigeru Tansho-Nagakawa, Chizuru Miyazaki, Kazuyuki Shimomura, Yasuo Ono, Shigeru Abe, Inhibition of neutrophil adhesion and antimicrobial activity by diluted hydrosol prepared from *Rosa damascena*. Biol. Pharm. *Bull Pharm Bull.*, 2017; 40(2): 161-168.
50. Najda A, Buczkowska H, Morphological and chemical characteristics of fruits of selected *Rosa* sp. *Modern Phytomorphol.*, 2013; 3: 99-103.
51. Sheng Y, Abreu IA, Cabelli DE, Maroney MJ, Miller AF, Teixeira M, Valentine JS, Superoxide dismutases and superoxide reductases. *Chem Rev.*, 2014; 114(7): 3854-3918.
52. Petropoulos S, Fernandes Â, Dias MI, Pereira C, Calhelha RC, Chrysargyris A, Tzortzakis N, Ivanov MD, Sokovic M, Barros L, Ferreira ICFR, Chemical composition and plant growth of *Centaurea raphanina* subsp. mixta plants cultivated under saline conditions. *Molecules*, 2020; 25(9): 2204: 1-26.
53. Priti SP, Pratima AT, Satish YG, *In vitro* antioxidant and free radical scavenging activity of extracts of *Rosa damascena* flower petals. *Am J Phytomed Clin Therap.*, 2015; 3(09): 589-601.
54. Rusu ME, Fizesan I, Pop A, Gheldiu AM, Mocan A, Crisan G, Vlase L, Loghin F, Popa DS, Tomuta I, Enhanced recovery of antioxidant compounds from hazelnut (*Corylus avellana* L.) involucre based on extraction optimization: phytochemical profile and biological activities. *Antioxidants (Basel)*, 2019; 8(10): 460: 1-27.
55. Sak K, Anticancer effects of flavonoids on melanoma cells: are murine cells more sensitive compared to humans?. *Int J Phytomed.*, 2013; 5(4): 441-445.
56. Schiber A, Mihalev K, Berardini N, Mollov P, Carle R, Flavonol glycosides from distilled petals of *Rosa damascena* Mill. *Z Naturforsch C J Biosci.*, 2005; 60(5-6): 379-384.
57. Gupta AK, Bharadwaj M, Mehrotra R, Skin Cancer Concerns in People of Color: Risk Factors and Prevention. *Asian Pac J Cancer Prev.*, 2016; 17(12): 5257-5264.
58. Shohayeb M, Abdel-Hameed ESS, Bazaid SA, Maghrabi I, Antibacterial and Antifungal Activity of *Rosa damascena* Mill. Essential Oil, Different Extracts of Rose Petals. *Global J Pharm.*, 2014; 8(1): 1-7.
59. Vicaș LG, Mureșan ME, Medicinal Plant Extracts. *Plants*, 2021; 10(5): 838: 1-2.
60. Sivaraj C, Abhirami R, Deepika M, Sowmiya V, Saraswathi K, Arumugam P, Antioxidant, antibacterial activities and GC-MS analysis of fresh rose petals aqueous extract of *Rosa damascena* Mill L. *J Drug Deliv Therapeut.*, 2019; 9(4-S): 68-77.
61. Sivasankarapillai VS, Madhu Kumar Nair R, Rahdar A, Bungau S, Zaha DC, Aleya L, Tit DM, Overview of the anticancer activity of withaferin A, an active constituent of the Indian ginseng *Withania somnifera*. *Environ Sci Pollut Res.*, 2020; 27(21): 26025-26035.
62. Stan RL, Sevastre B, Ionescu C, Olah NK, Vicaș LG, Páll E, Moisa C, Hanganu D, Sevastre-Berghian AC, Andrei S, Pripon-Furtună FR, Marcus I, Hangan AC, *Artemisia annua* L. extract: a new phytoproduct with SOD-like and antitumor activity. *Farmacia*, 2020; 68(5): 812-821.
63. Taneva I, Petkova N, Dimov I, Ivanov I, Denev P, Characterization of rose hip (*Rosa canina* L.) fruits extracts and evaluation of their *in vitro* antioxidant activity. *J Pharmacogn Phytochem.*, 2016; 5(2): 35-38.
64. Zahra M, Gholamreza A, Ghazaleh M, Mannan H, Comparative analysis of phenolic compounds in two samples of *Rosa damascena* by HPLC. *Int J Biosci.*, 2015; 7(1): 112-118.