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(RESEARCH ARTICLE)

# Pharmacognostic, phytochemical and pharmacological aspects of *Rosa x damascena* Herrm. Georgian cultivar

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## Abstract

*Rosa x damascena* Herrm. represents one of the most popular and important plants of the *Rosaceae* family. For ages, rose oil, rose water and extracts from its flowers were used in perfumery, cosmetics, aromatherapy, and medicine due to diverse pharmacological efficacy. Nowadays, special attention is paid to the valorization of wastes from the rose oil industry in order to fully recover potent biologically active compounds. The present study describes in detail the microstructural features of the flower petals and provides data on chemical composition and biological potency of the aqueous extract of rose oil waste from the *R. damascena* Georgian cultivar. Up to 25 constituents were detected by HPLC-MS in the extract, and the content of total flavonoids in the extract reaches 2.29%. Amongst them hyperoside is dominating, which content is not less than 1%. Pharmacological evaluation of the extract on "Hot plate" and carrageenan-induced oedema models in mice revealed analgesic (42%) and anti-inflammatory (67%) effects of the extract. As well, the extract revealed antiplasmodial activity against *Plasmodium falciparum* (3D7) strain (IC50 =20.31 µg/mL)

**Keywords:** Rosa x damascena Herrm; Microscopic characteristics; Flavonoids; Hyperoside; Biological activity

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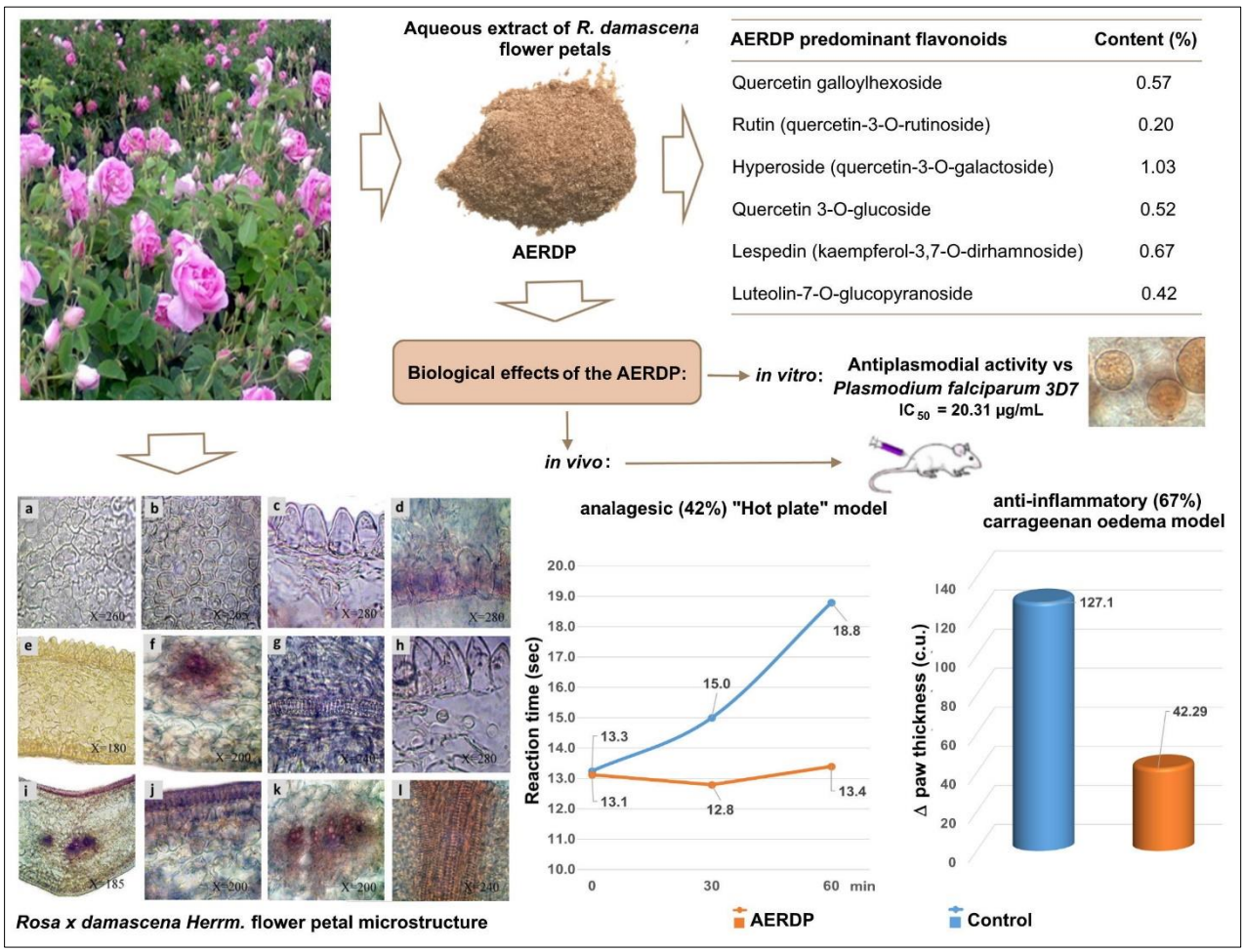
## Graphical abstract




## 1. Introduction

*Rosa x damascena* Herrm aka Damask rose is one of the most famous representatives of the genus *Rosa L.*, comprising up to 430 species [1], [2] and 18,000 cultivars [3]. Damask rose is introduced and cultivated in many countries mainly due to its great ornamental properties and the presence of valuable essential oil in the flower petals. From ancient ages, *R. damascena* essential oil is widely used as a valuable base material in perfumery, cosmetics, aromatherapy, and for different medicinal purposes [4]. The top producers of rose oil - Bulgaria, Turkey, Iran and India are currently supplying nearly 90% of this product to the world market [5], [6], [7].

The chemical composition of essential oils significantly varies due to diverse climatic conditions and soil composition of *R. damascena* plantations [8], [9], [10], [11], [12]. Other factors that contribute to the variability of major constituents and yield of the oil are different techniques and/or conditions of the extraction process [10], [13], [14]. Due to minor content of oil in rose flowers (0.030–0.045%), the industrial process, especially the one based on hydro distillation method, is accompanied by the formation of large amounts of waste (solid residues and waste water. Most often, this waste was simply thrown away without any further processing, though in past decade several methods for valorization of rose waste became available [15], [16]. On the other hand, the same waste contains a sufficient amount of biologically active compounds, mainly polyphenols, flavonoids and polysaccharides [17], [18], [19], [20], [21], [22]. *R. damascena* hydroalcoholic and aqueous extracts are claimed to be responsible for diverse pharmacological properties of R. *damascena:* antioxidant [17], [18], [23], [24], [25], analgesic [26], anti-inflammatory [24], [27], hypnotic [28], memory-enhancing [29], anticonvulsant [30]. The investigation both of rose oils and its by-products obtained from plants growing in different regions is yet in progress.

In recent years industrial plantations of Damask rose have been established in Kakheti floristical region of Georgia (Fig. 1). In present investigation, we attempted to analyse some microscopic, phytochemical and pharmacological features

of *R. damascena* Georgian cultivar with particular attention to chemical composition of the waste left after the obtaining rose oil by hydrodistillation method.


**Figure 1** Plantations of *Rosa x damascena* Herrm. a – general view; b – Kakheti floristical region of Georgia (highlighted in red)

## 2. Material and methods

### 2.1. Plant material

The plant material (flower crown petals of cultivated *Rosa damascena*) was collected from Kakheti floristical region of Georgia during the active flowering season in May, 2020 (Fig. 1). The identification of the plant was carried out in the Department of Botany at Iovel Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University. A voucher specimen (TBPH–21167) was deposited in the herbarium of the named institute. Transverse, longitudinal, and superficial cross sections from central areas of non-fixed fresh petals were immersed in the saffron solution for 24 h and then placed on a glass slide with a drop of glycerin. Sections were observed with Jeneval (Carl Zeiss, Germany) light microscope and documented using attached digital camera. Finally, the digital images were post-processed using Adobe Photoshop CS5 software.

### 2.2. Chemicals and Reagents

Acetonitrile HPLC grade was purchased from Merck. & Co. All other reagents, and solvents, and chemicals used in the experiments were of analytical grade and obtained from Sigma Aldrich (USA). Ultrapure water for HPLC analysis was produced by Millipore Classic Purification System.

### 2.3. Preparation of aqueous extract of *R. damascena* flower petals (AERDP)

*R. damascena* flower petals were processed by hydrodistillation according to the standard procedure described in the European Pharmacopoeia [31]. The waste water was concentrated using rotary vacuum evaporator below 40ºC. The extract was frozen in a layer of 10 mm in the Petri dishes at -20 °C in freezer for 12 h, then vacuum dried at -90 °C under 3.33 Pa absolute pressure to constant weight. The dried material was powder-grinded and stored in vacuum desiccator until further use.

### 2.4. Determination of total flavonoid content in AERDP

Total flavonoid content (TFC) of AERDP was determined as prescribed in BP 2016 [32].

The TFC was expressed as hyperoside equivalent (HE) and calculated as using the following expression: A x 1.235/m, where A is absorbance at 410 nm; m - mass of the extract to be examined, in grams.

### 2.5. HPLC analysis of AERDP

The separation of the compounds was performed on analytical column Eclipse plus C-18 (4.6 x 250 mm; 5 µm) using an Agilent 1260 Infinity II LC System (Agilent, USA), equipped with G4225A degasser, G1312 binary gradient pump, G1329 autosampler, G1316A column oven, and G4212B diode array detector.

Chromatography conditions: t - 25°C; mobile phase - 0.1% (v/v) formic acid in water (eluent A) and of 0.1% (v/v) formic acid in acetonitrile (eluent B); gradient program - 10% B to 45% B (50 min), 10% B to 100% B (10 min), 100% B (5 min); flow rate - 0.5 ml/min. The injection volume for all samples was 10 µl. Detection wavelength - at 254 nm. Spectra were recorded from 200 nm to 400 nm.

LC-MS analysis was performed with the HPLC system described above connected in series with an Agilent Technologies 6420, Triple Quad LC/MS fitted with an ESI source. Negative ion mass spectra of the column eluate were recorded in the range m/z 100-1500. Nitrogen was used as the dry gas at a flow rate of 11 l/min. The process was controlled by Agilent MassHunter software.

Hyperoside (quercetin 3-O-galactoside), rutin (quercetin-3-O-rutinoside), lespedin (kaempferol 3, 7-O-dirhamnoside), and quercetin 3-O-glucoside were used as standards in HPLC and MS analysis.

### 2.6. Preparation of standard solution

Standard solution of hyperoside was prepared in methanol to a final concentration of 1.0 mg/ml. A series of working solutions of hyperoside (n=5) was prepared in order to obtain various concentration levels (0.002-1.0 mg/ml). The appropriate volume of solution of hyperoside was introduced in a 10.0 mL volumetric flask and the volume was adjusted to 10.0 mL with methanol. All prepared standard solutions were filtered through 0.45 µm membrane filter (Millipore, ref HVPL04700) before HPLC analysis.

### 2.7. Preparation of sample solution

100.0 mg of the AERDP was introduced in a 10 mL volumetric flask and solubilized in methanol and the volume was adjusted to 10.0 mL with methanol. Then 2 ml of solution was filtered through a syringe filter (0.45 mm Millipore) into a HPLC vial.

### 2.8. Quantification of individual compounds

Hyperoside was quantified using a calibration curve of the corresponding standard. Sample preparation and HPLC determination were performed in duplicate. Data are expressed as mean± standard deviation.

### 2.9. Biological experiments

#### *2.9.1. Animals*

Inbred white mice weighing 28 ± 2 g (n = 40) were used. Animals were obtained from the animal house of Tbilisi State Medical University I. Kutateladze Institute of Pharmacochemistry and quarantined for 1 week in the Department of Preclinical Pharmacological Research of above Institute under standard conditions (temperature 20 ± 2°C, humidity 55-65%, 12/12-hour light/darkness cycle, granulated food - 4 g/animal/day, water ad libitum) prior to experiment. All experiments were carried out in accordance with the EU Directive 2010/63 and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health requirements, and approved by the Tbilisi State Medical University Ethics Committee on Animal Research (registration number AP-52-2021).

#### *2.9.2. Analgesic activity (Hot plate assay)*

The animals were individually placed in an open cylindrical space consisting of a metal floor heated to a temperature of 52 ± 2°C and transparent vertical walls. The time between the placement of the animal on the floor and the first nociceptive reaction (hind paw licking or jumping) was recorded as the hot-plate latency. Measurements were done

before the intraperitoneal administration of the 100 mg/kg AERDP (baseline latency) and subsequently every 30 minutes for one hour. Mice with baseline latencies higher than 10 s were eliminated from the study. The analgesic effect was calculated by the formula: $E\% = ((T_0-T_n) /T_0) \times 100$, where $T_0$ is the reaction time prior to the AERDP injection, and $T_n$ - after the corresponding period (30 or 60 min) after injection, respectively [33].

*2.9.3. Anti-inflammatory activity (Carrageenan induced paw oedema assay)*

50 µl of 1% carrageenan solution in saline was injected in the aponeurosis of the right hind paw of the animal. One hour prior the onset of oedema, 0.5 ml of saline and 0.5 ml of AERDP at a dose of 100 mg/kg are administered intraperitoneally to control and experimental animals, respectively. The thickness of the paw was measured with a digital micrometer before the carrageenan injection (baseline) and after 2 hours. Anti-inflammatory efficacy was calculated by the following formula: $E\%= (1-(\Delta T_{exp} /\Delta T_{con})) \times 100$, where $\Delta T_{con}$ and $\Delta T_{exp}$ are the mean differences in paw thickness before and 2 hours after carrageenan administration in control and experimental group animals, respectively [34].

*2.9.4. Antiplasmodial assay*

The culture of chloroquine-sensitive strain of *Plasmodium falciparum* (3D7 was obtained through BEI Resources, NIAID, and NIH: Plasmodium falciparum, Strain 3D7, MRA-102, contributed by Daniel J. Carucci. The AERDP was evaluated *in vitro* as previously described [35], [36]. Artemisinin (98%, Sigma– Aldrich) was used as antimalarial reference compound ($IC_{50}$ 0.00425 ± 0.008 µM). The results were expressed as the mean $IC_{50}$ (the concentration of a drug that reduced the level of parasitemia by 50%). All tests were performed in triplicate.

*2.9.5. Statistical analysis*

Statistical analysis of the experimental data was performed using Student's t-test [37]. Statistical significance was set at $p \leq 0.05$.

## 3. Results and discussion

### 3.1. Microscopic characteristics of *R. damascena* petals

The underlying stitched cells of the petals of the *R. damascena* abaxial epidermis belong to the curvilinear clan, curvilinear type (Fig. 2-a) whereas the underlying cells of the adaxial epidermis are partially arranged, belonging to the rectangular clan, oblique type (Fig. 2-b). The structural elements of the upper epidermis of the flower crown petal have a thick structure. *R. damascena* flower petal is bifacial, bare. The covering tissue on both sides of the petal is single-layered, in the ventral plane there is a mastoid epidermis (Fig. 2-c), and in the dorsal - a linear, dense, weakly toothed-cuticular structure (Fig. 2-d)

Parenchyma is amorphous, represented by loosely arranged thin-skinned, blunt-angled heterogeneous parenchymal cells (Fig. 2-e). Secretory paths in the form of two nested contoured rings are observed mostly in the ventral part of the petal parenchyma (Fig. 2-h). More or less intensively fixed small, amphicribral-type conductive cones (Fig. 2-f) with spirally thickened tracheid's (Fig. 2-g) are observed in petal parenchyma as well.

The main conductive vessel of the *R. damascena* petal is bordered by conical, dull epidermis on the adaxial part, and by straight, angular, single-layered, tight cutinized protective tissue on the abaxial (Fig. 2-i). In the ventral and dorsal planes of the main vessel texture, 2 layers of atypical palisade parenchymal cells are observed (Fig. 2-j), including heterogeneous tissue consisting from loose, predominantly large, single-layered integumentary cells around the reverse-collateral type conductive bundle (Fig. 2-k). In the vascular bundle timber, small-caliber spirally thickened, round lumens are observed (Fig. 2-l).

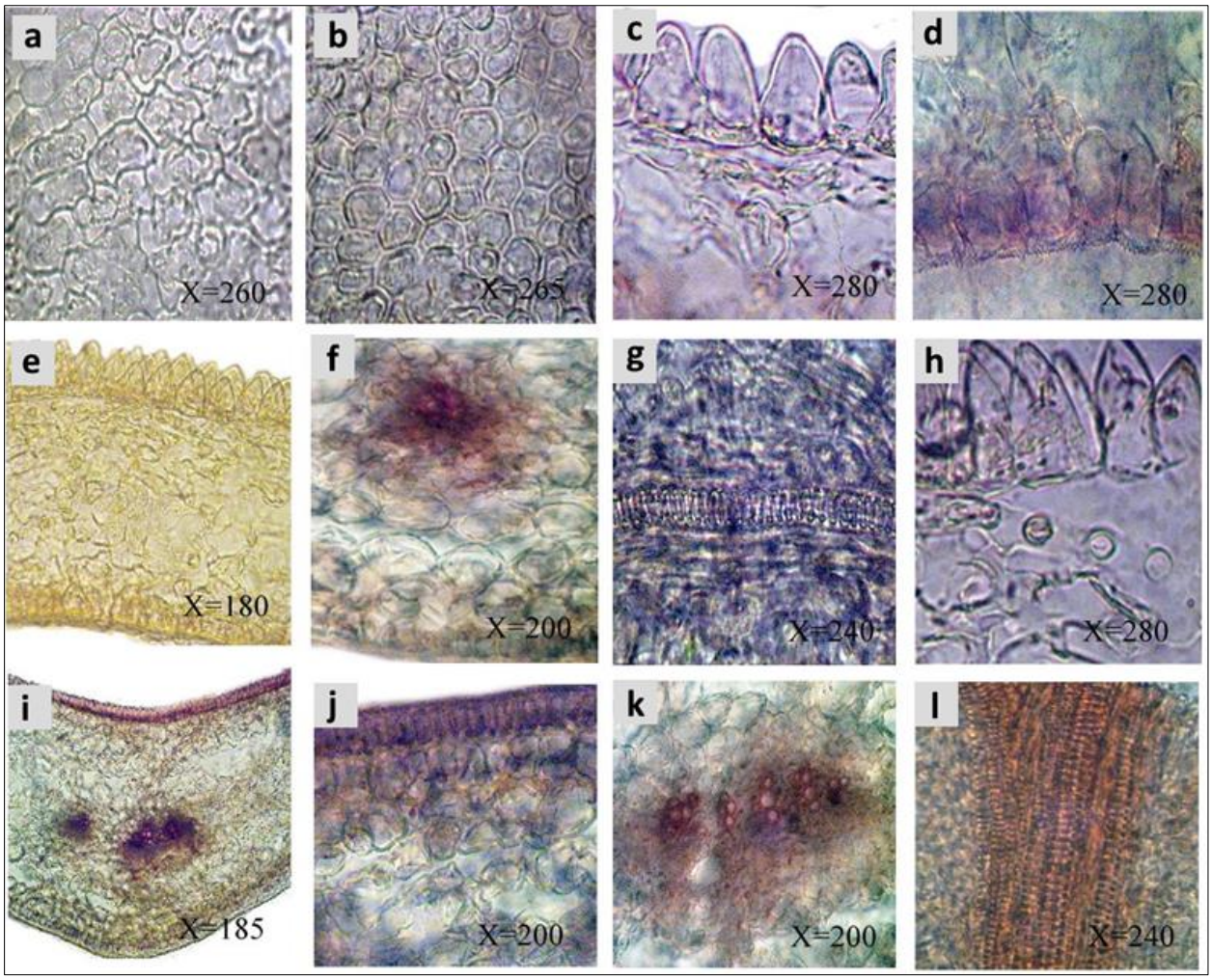



**Figure 2** *Rosa × damascena* Herrm. flower petal microstructure: a - abaxial epidermis; b - adaxial epidermis; c - squamous cells; d - toothed-cutinized cells; e - petal parenchima; f - amphibrillar conductive bundle; g - spiral anastomosis; h - secretory pathway; i - main vessel; j - atypical palisade parenchyma; k - reverse-collateral conductive bundle; l - spirally encapsulated timber vessels

### 3.2. Flavonoid content of AERDP

HPLC elution conditions were optimized in order to achieve satisfactory separation [38].

**Table 1** Characteristic data and content of AERDP flavonol glycosides

| N | Compound | RT | DAD absorbance maximums (nm) | (M-H) | Content (%) |
|---|---|---|---|---|---|
| 1 | Quercetin galloylhexoside | 21.1 | 231, 256, 265, 302, 353 | 615 | 0.57 |
| 2 | Rutin (quercetin-3-O-rutino-side) | 23.1 | 231, 256, 275, 310, 353 | 609 | 0.20 |
| 3 | Hyperoside (quercetin 3-O-galactoside) | 24.3 | 231, 256, 280, 310, 353 | 463 | 1.03 |
| 4 | Quercetin 3-O-glucoside | 24.6 | 231, 256, 280, 310, 355 | 463 | 0.52 |
| 5 | Lespedin (kaempferol 3,7-O-dirhamnoside) | 27.3 | 232, 265, 300, 348 | 593 | 0.67 |
| 6 | Luteolin-7-O-glucopyranoside | 28.6 | 231, 256, 280, 310, 353 | 447 | 0.42 |

The optimal separation of the AERDP was achieved on the reverse phase column Eclipse plus C-18 (4.6 x 250 mm; 5 μm) operated at 25°C. To provide an optimal resolution, the effect of mobile phase was examined. A best separation was detected under the following conditions: mobile phase 10% B to 45% B (50 min), 10% B to 100% B (10 min), 100% B (5 min). 25 major compounds were detected. Among them the dominant flavonoids - hyperoside (quercetin 3-O-galactoside), rutin (quercetin-3-O-rutinoside), lespedin (kaempferol 3, 7-O-dirhamnoside), quercetin 3-O-glucoside and quercetin galloylhexoside have been identified using reference standards and MS data (Table 1). The main constituent of AERDP was hyperoside (quercetin-3-O-galactopyranoside), which content in all series was not less than 1%.

### 3.3. Quantification of the AERDP

HPLC separation conditions were optimized in order to achieve satisfactory resolution. A baseline separation of the AERDP was achieved within 25 min, with symmetrical, sharp and well resolved peak for hyperoside. This method was validated according to the ICH guidelines on the validation of analytical methods. No interference from other compounds but great resolution was observed for hyperoside. The peak purity was verified using Agilent ChemStation software. Hyperoside calibration curve was linear within the concentration range 0.0625-1.0 mg/ml. All correlation coefficients were greater than 0,999. The intraday precision (% RSD) was less than 1.7 % and the intraday precision (%RSD) was less than 1.04% evidencing a good precision of the method. Recoveries determined for hyperoside ranged from 97.5 to 101.5% and RSDs were less than 1.03% indicating a good accuracy (Fig. 3)

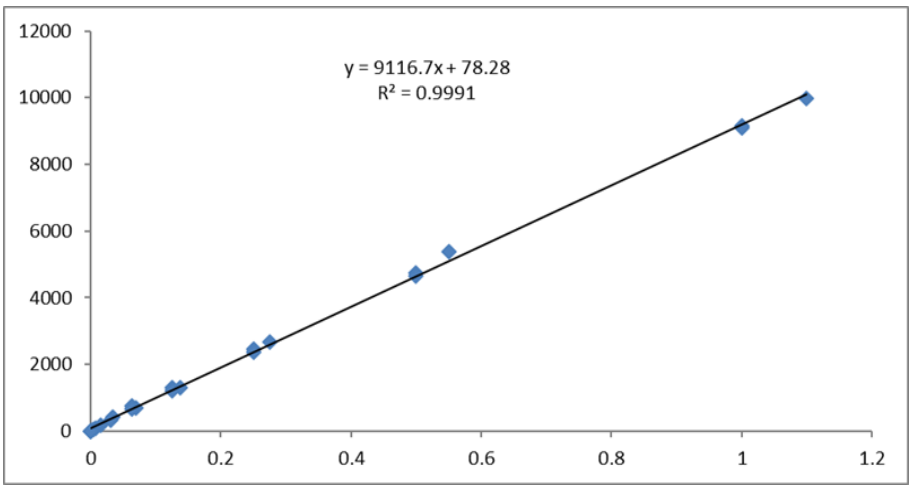



**Figure 3** Hyperoside calibration curve. Y-axis – peak area; X-axis – concentration (mg/ml)

### 3.4. Biological experiments

Flavonoids, one of the most diverse and widespread groups of plant secondary metabolites, are well known for having a broad spectrum of biological activity, e.g., anti-inflammatory, anticarcinogenic, antidepressant, antibacterial, antimutagenic, anti-HIV, and the most investigated free radical scavenging and antioxidative properties [39], [40]. Due to generally known ability of quercetin glycosides to suppress the production of pro-inflammatory factors, such as cyclooxygenase (COX) and lipoxygenase (LOX), cytokines IL-1β, TNF-α, IFN-γ, and NO [41], [42], [43] we did not repeat these assays and focused on in vivo experiments. In particular, we aimed to estimate anti-inflammatory, antinociceptive and antiparasitic efficacy of the AERDP.

#### *3.4.1. Carrageenan oedema assay*

In the experimental group animals, 2 hours after the carrageenan administration, an increase in the thickness of the paw was 48.8±7.3 μm, which was 2.5 times less than in control group animals (124.5±6.8 μm). The efficacy of AERDP was 61% at 2 h ($p<0.01$), evidencing a pronounced anti-inflammatory activity. Our results correlate with literary data [44], [45], [46], [47], [42], [43], thus confirming the anti-inflammatory potential of aqueous hyperoside-containing extracts.

#### *3.4.2. Hot plate assay*

The hot-plate latency in the experimental group was 13.3±2.2 seconds before the administration of 100 mg/kg AERDP, 15.0±5.3 and 18.8±4.7 seconds on the 30$^{th}$ and 60$^{th}$ minutes after the injection, respectively; in the control animals the hot-plate latency was 13.1±2.6, 12.8±3.0 and 13.4±2.8 seconds, respectively. The maximal analgesic effect of the AERDP

was 42% ($p<0.05$). Hence, the AERDP provides moderate short-term analgesia that confirms data [42], [43], [45] on the analgesic effect of extracts similar in flavonoid composition.

*3.4.3. Antiplasmodial assay*

AERDP was evaluated against *Plasmodium falciparum* (3D7) and revealed effective antiplasmodial activity ($IC_{50}$ = 20.31 µg/mL). To our knowledge, such activity of the aqueous extract of rose oil waste from the *R. damascena* Georgian cultivar was not previously described.

## 4. Conclusion

The present study provides data on chemical composition and biological potency of the aqueous extract of rose oil waste from the *R. damascena* Georgian cultivar. Based on the results obtained, it can be concluded that the waste of rose oil contains valuable amounts of bioactive substances (glycosides of quercetin and kaempferol) that can be used in the pharmaceutical and cosmetic industry as main or auxiliary components. Apart from the recovery of valuable substances, more complete utilization of the rose oil production waste will help to reduce the total amount of generated waste that is very important from an ecological viewpoint. Hence, studies in this direction should be continued.

## Compliance with ethical standards

### *Acknowledgments*

The authors are thankful to the Tbilisi State Medical University Iovel Kutateladze Institute of Pharmacochemistry for the support and express their gratitude to Pirose LTD for supplying the raw plant material for this study.

### *Disclosure of conflict of interest*

The authors declare no financial or any other conflicts of interest in this work.

### *Statement of ethical approval*

The animal research protocol was approved by the Tbilisi State Medical University Ethics Committee on Animal Research (registration #AP-52-2021).

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