

(RESEARCH ARTICLE)



## Phytochemical and pharmacological study of *Rosa x gallica* L. georgian cultivar essential oil production waste

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GSC Biological and Pharmaceutical Sciences, 2022, 19(03), 213–222

Publication history: Received on 15 May 2022; revised on 16 June 2022; accepted on 18 June 2022

Article DOI: <https://doi.org/10.30574/gscbps.2022.19.3.0242>

### Abstract

The study aimed to assess chemical composition and pharmacological properties of *Rosa x gallica* cultivated in Georgia for application in medicine and cosmetology.

The quantitative and qualitative composition of secondary metabolites of the aqueous extract obtained during the production of essential oil of *R. gallica* (AERGP) was analysed using HPLC analytical methods, and its pharmacological activity was evaluated in *in vitro* and *in vivo* experiments.

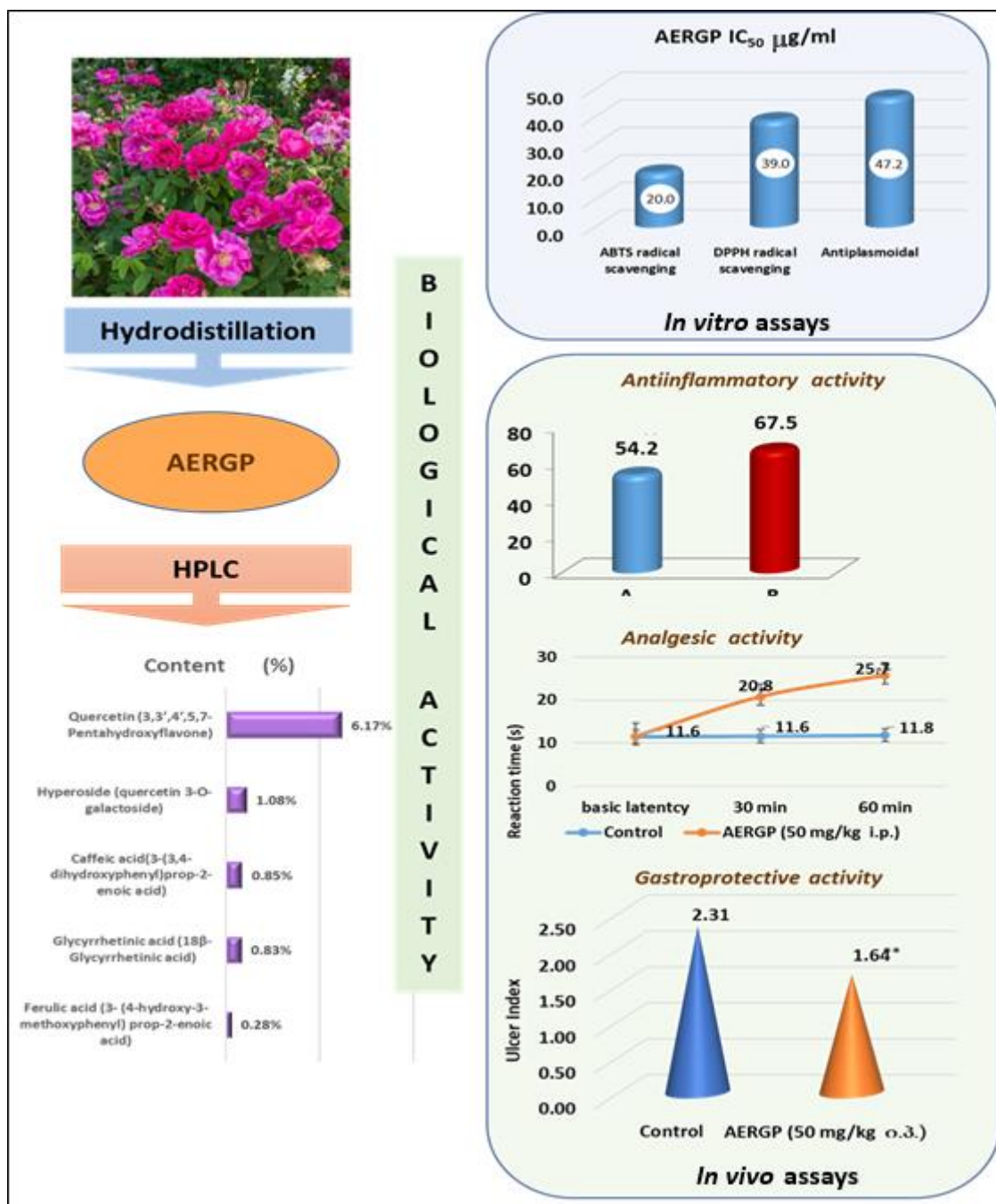
Five prevalent compounds have been established in AERGP: Glycyrrhetic acid (0.83%), Hyperoside (1, 08%), Caffeic acid (0, 85%), Quercetin (6, 17%), and Ferulic acid (0, 28%). Studies of antioxidant activity of AERGP revealed its dose-dependent radical scavenging effect in both ABTS (IC<sub>50</sub> 20.0 µg/mL), and DPPH (IC<sub>50</sub> 39.0 µg/mL) assays. AERGP appeared active against *Plasmodium falciparum* 3D7 strain (IC<sub>50</sub> 47.2 µg/mL). In *in vivo* experiments AERGP showed pronounced analgesic (124%) and anti-inflammatory effects (67.58%), as well as a moderate gastroprotective efficacy (28, 9%).

**Keywords:** *Rosa x Gallica*; Flavonoids; Quercetine; Anti-Inflammatory; Analgesic; Antioxidants

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## Graphical Abstract



## 1. Introduction

In tandem with the development of the global economy and industry the volume of industrial waste is expanding that is a contemporary challenge. Developed countries around the world are attempting to limit the amount of industrial waste by valorizing it, which leads to a more complete recovery of valuable substances.

The Rosacea family contains up to 3200 species from 115 genera. From ancient times roses were cultivated for ornamental and medicinal uses by the Romans, Greeks, and Persians and nowadays over 35000 varieties are obtained by hybridization and selection from Tea rose (*R. chinensis*) and the deciduous large-flowered *R. damascena* [1]. Since 1948, scientific research has been carried out on rose introduction and hybrid selection in Georgian botanical gardens [2].

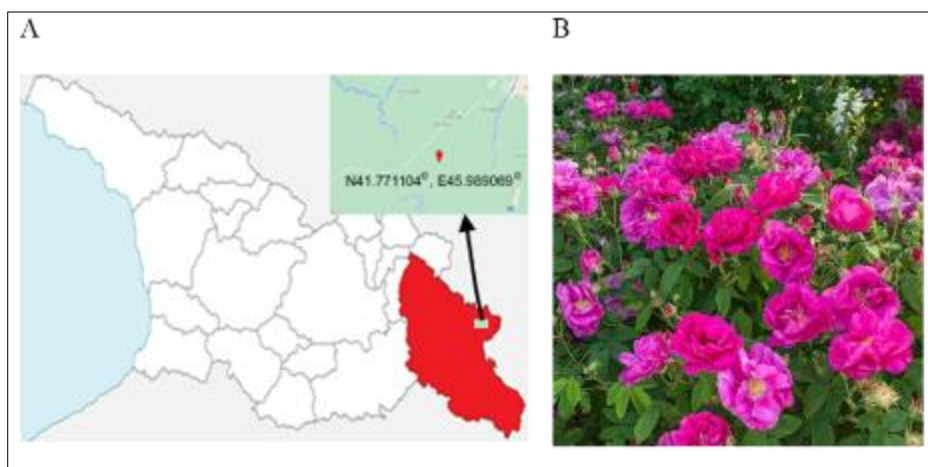
Phytochemical studies have shown that rose petals contain essential oil, anthocyanins, terpenes, flavonoids and other secondary metabolites [3]. Flavonoids have strong anti-inflammatory, analgesic and antioxidant effects [4], they suppress inflammation, increase immunity, inhibit cell growth signaling pathways such as mTOR and Ras oncogenic pathways, resulting in inhibition of the cell cycle [5]. Geraniol provides apoptosis of tumor cells [6]. Anthocyanins are claimed to suppress natural oxidative process, to protect the body from neurodegenerative and cardiovascular diseases [7] and have antidiabetic effects [8]. Cosmetology studies have confirmed *R. x gallica*'s effects on skin rehydration, minimizing the production of age-related wrinkles, nourishing the epidermis, improving skin elasticity, and having a rejuvenating impact [9].

The current study aimed to explore the phytochemical composition of the aqueous extract obtained during the production of essential oil from *R. gallica* grown in Georgia, as well as to assess its pharmacological action for prospective use in medicine and cosmetics.

## 2. Material and methods

### 2.1. Chemicals and Materials

Raw material for the study - flower crown petals of cultivated *Rosa x gallica* collected in May, 2021, was contributed by Pirose LTD Kakheti region of Georgia (Fig. 1). All analytical grade reagents, solvents and chemicals used in the experiments were purchased from Sigma Aldrich (USA), except for HPLC grade acetonitrile (Merck. & Co). Millipore Classic Purification System was used to obtain ultrapure water for HPLC analysis. The strain of chloroquine-sensitive *Plasmodium falciparum* strain 3D7, was obtained through MR4-BEI Resources, NIAID, NIH: *Plasmodium falciparum* strain 3D7, MRA-102, was contributed by Daniel J. Carucci.



**Figure 1** A - Georgia, Kakheti region (highlighted in red); B - general view of *Rosa x gallica* L

### 2.2. Preparation of aqueous extract of *R. gallica* petals (AERGP)

Hydrodistillation of *R. gallica* flower petals was performed in accordance with the European Pharmacopoeia (Ph. Eur. 2008) guideline [10]. A rotary vacuum evaporator was used to concentrate the waste water below 40 °C. The extract was freeze-dried in a layer of 10 mm on Petri dishes at -20 °C for 12 hours, brought to constant weight at -90 °C under 3.33 Pa. Finally, it was powder-grinded and stored in a vacuum desiccator until it was needed again.

### 2.3. Determination of total flavonoid content in AERGP

The total flavonoid content (TFC) of AERGP was measured according to the British Pharmacopoeia 2017 [11]. The following formula was used to calculate TFC as Quercetin equivalent (QE):  $A \times 1.235/m$ , where A is the absorbance at 410 nm and m - the mass of the extract in grams.

### 2.4. HPLC analysis of AERGP

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 and controlled by Agilent MassHunter software. Negative ion mass spectra of the column eluate were recorded in the m/z range 100-1500. Nitrogen was used as the dry gas at a flow rate of 11 l/min. Glycyrrhetic acid (18β-Glycyrrhetic acid), hyperoside (quercetin 3-O-galactoside), caffeic acid(3-(3,4-dihydroxyphenyl) prop-2-enoic acid), quercetin (3,3',4',5,7-Pentahydroxyflavone) and ferulic acid (3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid) were used as standards in HPLC and MS analysis.

## 2.5. Preparation of standards and sample solution

Standard solution of quercetin was prepared in methanol to a final concentration of 1.0 mg/ml. A series of working solutions of quercetin (n=5) was prepared in order to obtain various concentration levels (0.002-1.0 mg/ml). The appropriate volume of solution of quercetin 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. 100.0 mg of the AERGP was solubilised in a 10 mL volumetric flask with methanol. Then 2 ml of solution was filtered through a syringe filter (0.45 mm Millipore) into a HPLC vial for the analysis.

## 2.6. Quantification of individual compounds

Quercetin 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.

## 2.7. Biological experiments

### 2.7.1. Scavenging Activity of ABTS Radical

The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) scavenging activity of AERGP was determined according to a literature method with slight modifications [14],[15]. The radical cation ABTS<sup>•+</sup> was generated by persulfate oxidation of ABTS. A mixture of ABTS (0.7 mM) and sodium persulfate (2.45 mM) was realized and kept overnight at room temperature in the dark to form a radical cation ABTS<sup>•+</sup>, for a further use. Then, the solution was diluted 200 times with methanol and 1.980 mL was transferred into a 5-mL tube at which 20µL of the tested samples were added at the final concentrations of 5 to 40 µg/mL respectively. The resulting solution was mixed using a vortex and stored at room temperature in the dark for 1 h. then the absorbance of solutions was measured at 734 nm. The antioxidant activity was calculated by equation % inhibition = [(Ac - As)/Ac] × 100, where Ac and as is absorbance of control and sample solutions, respectively.

### 2.7.2. Scavenging Activity of DPPH Radical

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical-scavenging activity was determined as described in [16],[17] with minor modifications. The Vc (DPPH control concentration) was 0.625mg/mL. Serial dilutions of AERGP solution (3.125; 6.25; 12.5; 25 and 50 µg/ml) and Vc were placed in 96 well microtiter plates. Then, 20 µL of DPPH free radical agent (0.0158 g dissolved in 50 mL 95% (v: v) ethanol) and 95% (v: v) etha nol were added to a total volume of 200 µL and incubated at 37°C for 30 min. After the incubation the absorbance was read at 540 nm. The antioxidant activity was calculated as follows: %inhibition = [(Ac - As)/Ac] × 100, where Ac and as is absorbance of negative control and sample after 30 min, respectively.

### 2.7.3. Antiplasmodial activity

The activity of AERGP against *Plasmodium falciparum* strains was evaluated *in vitro* as previously described [12], [13] using Artemisinin (98%, Sigma– Aldrich) as reference compound (IC<sub>50</sub> 0.0043 ± 0.008 µM). The results were expressed as the mean IC<sub>50</sub> (the concentration of a drug that reduced the level of parasitaemia by 50%).

### 2.7.4. Animals

Inbred CD-1 mice in the weight range of 24-28 g (n = 40) were used in all experiments. Animals were kept in the Tbilisi State Medical University I. Kutateladze Institute of Pharmacochimistry Department of Preclinical Pharmacological Research under standard conditions (room temperature 20 ± 2°C, humidity 55-65%, 12/12-hour light and dark cycle, granulated food - 4 g/animal/day, water ad libitum). All experiments adhered to EU Directive 2010/63 and the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health requirements. The research protocol was

submitted to and approved by the Tbilisi State Medical University Ethics Committee on Animal Research (registration number AP-56-2022).

#### 2.7.5. Analgesic activity (Hot plate assay)

The AERGP was administered to test group animals (n=10) intraperitoneally at a dose of 100 mg/kg. The control group (n=10) was intraperitoneally exposed to a normal saline (0.4 ml/animal). Mice were placed individually in the transparent vertical cylindrical chamber with a metal floor heated to  $52\pm 2^\circ\text{C}$ . The hot-plate latency was determined by the time between placing the animal on the floor and the first nociceptive reaction (hind paw licking or jumping). The measurements were taken before the AERGP/saline injection (baseline latency), 30 minutes, and one hour afterwards. Mice with baseline latencies greater than 15 seconds were excluded 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 AERGP injection, and  $T_n$  - after the corresponding period (30 or 60 min) after injection, respectively [18],[19].

#### 2.7.6. Anti-inflammatory activity (Carrageenan induced paw oedema assay)

Mice were divided into groups (control and AERGP-treated) with each group containing 6 animals. Acute inflammation was induced by subplantar injection of 50  $\mu\text{l}$  of 1% carrageenan solution in normal saline was injected in the right hind paw of mice. One hour prior the carrageenan injection, 0.5 ml of normal saline and 0.5 ml of AERGP at a dose of 100 mg/kg were 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 expressed as percentage inhibition of the inflammation was calculated by the following formula:  $E(\%) = (1 - (\Delta T_{\text{exp}} / \Delta T_{\text{con}})) \times 100$ , where  $\Delta T_{\text{con}}$  and  $\Delta T_{\text{exp}}$  are the mean differences in paw thickness before and 2 hours after carrageenan administration in control and experimental group animals, respectively [20].

#### 2.7.7. Gastroprotective activity (Ethanol induced ulcer model)

The experiment was carried out as described by Adinortey et al. [21]. In brief, 18 outbred mice were randomly distributed in three groups of animals, each consisting of six mice. Food intake was restricted 24 hours before the experiment, and the animals were transferred to cages with a wide wire mesh raised floor to prevent coprophagy. To avoid excessive dehydration during the fasting period, all mice had free access to a nutritive solution of 8% sucrose in 0.2% NaCl. On day two in a dose of 50 mg/kg or normal saline (0.4 ml/animal) was given intraperitoneally to test and control mice, respectively. Absolute ethanol was given orally (1 ml/100 g) to all animals after AERGP/saline administration. 1 hour after the ethanol administration mice were euthanized by  $\text{CO}_2$  inhalation. The stomachs were immediately removed, opened along the great curvature, rinsed consequently with water and 10% formalin solution which contains about 4% formaldehyde w/v, fixed on white polystyrene board, and digitally photographed. Ulcer index (UI) was calculated for each stomach according to the following scale, by three independent observers: 1 - no lesions; 2 - single petechial lesions; 2.5 - multiple petechial or short linear hemorrhagic lesions; 3 - long linear hemorrhagic lesions; 4 - continuous linear hemorrhagic lesions along the entire length of the glandular part of stomach. The efficacy of AERGP expressed as percentage of ulcer inhibition (% I) was estimated based on the UI and calculated using the formula:

$$\% I = \frac{UI_C - UI_T}{UI_T} \times 100$$

Where  $UI_C$  and  $UI_T$  are macroscopic ulcer indexes in control and test groups, respectively.

#### 2.7.8. Statistical analysis

All tests were performed in triplicate. Statistical analysis of the experimental data was performed using Student's t-test [22]. Statistical significance was set at  $p \leq 0.05$ .

## 3. Results and discussion

### 3.1. Flavonoid content of AERGP

HPLC elution conditions were optimized in order to achieve satisfactory separation [23]. The optimal separation of the extract was achieved on a reverse phase column Eclipse plus C-18 (4.6 x 250 mm; 5  $\mu\text{m}$ ) operating at  $25^\circ\text{C}$ . 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

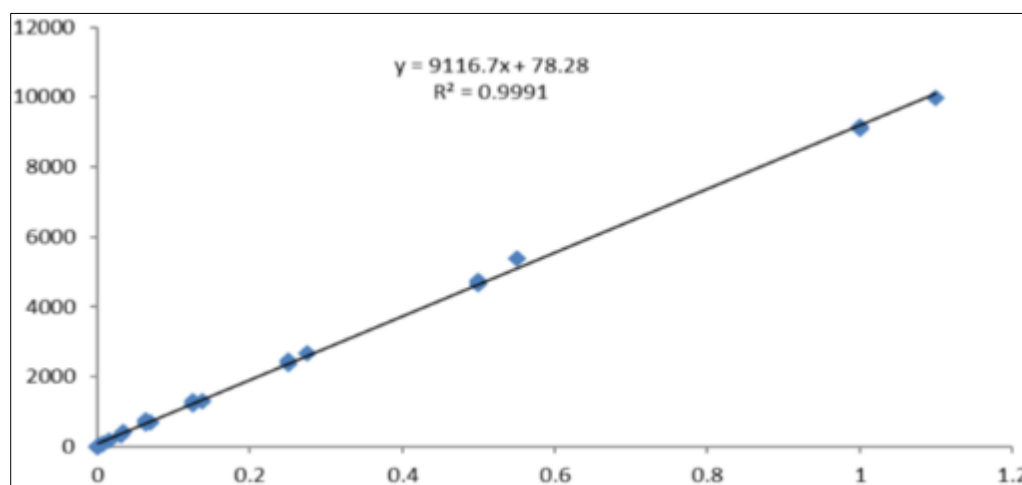
were detected. Among them, the dominant compounds - Glycyrrhetic acid (18 $\beta$ -Glycyrrhetic acid), Hyperoside (quercetin 3-O-galactoside), Caffeic acid(3-(3,4-dihydroxyphenyl) prop-2-enoic acid), Quercetin (3,3',4',5,7-Pentahydroxyflavone) and Ferulic acid (3- (4-hydroxy-3-methoxyphenyl) prop-2-enoic acid) have been identified using reference standards and MS data (Table 1). The main constituent of AERGP was quercetin (3,3',4',5,7-Pentahydroxyflavone), which content in all series was not less than 1%.

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

N	Compound	RT	DAD absorbance maximums (nm)	Content (%)
1	Glycyrrhetic acid (18 $\beta$ -Glycyrrhetic acid)	12.25	205, 210	0.83%
2	Hyperoside (quercetin 3-O-galactoside)	11.01	231, 256, 280, 310, 353	1.08%
3	Caffeic acid(3-(3,4-dihydroxyphenyl) prop-2-enoic acid)	8.85	210, 240, 295, 325	0.85%
4	Quercetin (3,3',4',5,7-Pentahydroxyflavone)	11.12	220, 255, 280, 310, 355	6.17%
5	Ferulic acid (3- (4-hydroxy-3-methoxyphenyl) prop-2-enoic acid)	11.88	235, 280, 322	0.28%

### 3.2. Quantification of the AERGP

To attain sufficient resolution, the HPLC separation parameters were optimized. Within 25 minutes, the AERGP had a baseline separation, with a symmetrical, crisp, and highly resolved peak for hyperoside. This method was validated in accordance with the ICH criteria for analytical method validation. There was no interference from other chemicals, while quercetin had excellent resolution. Agilent ChemStation software was used to verify the peak purity. Within the concentration range of 0.0625-1.0 mg/ml, the quercetin calibration curve was linear. All correlation coefficients exceeded 0,999. The intraday precision (%RSD) was less than 1.04% evidencing a good precision of the method. The recoveries for quercetin ranged from 98.0 to 101.2% with RSDs were smaller than 1.03%, indicating a good accuracy (Fig. 2)



**Figure 2** Quercetin calibration curve. Y-axis – peak area; X-axis – concentration (mg/ml)

### 3.3. Biological experiments

AERGP appeared to be rich in flavonoids, one of the most widespread groups of plant secondary metabolites with diverse, biological activity [24],[25],[26]. Considering the well-known ability of quercetin glycosides to suppress the production of pro-inflammatory factors, such as cyclooxygenase (COX) and lipoxygenase (LOX), cytokines IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , and NO [15], [26], [27] we gave preference to *in vivo* experiments and focused our attention on antioxidant, anti-inflammatory, analgesic, gastroprotective, and antiparasitic efficacy of the AERGP.



### 3.3.1. ABTS and DPPH radical scavenging assay

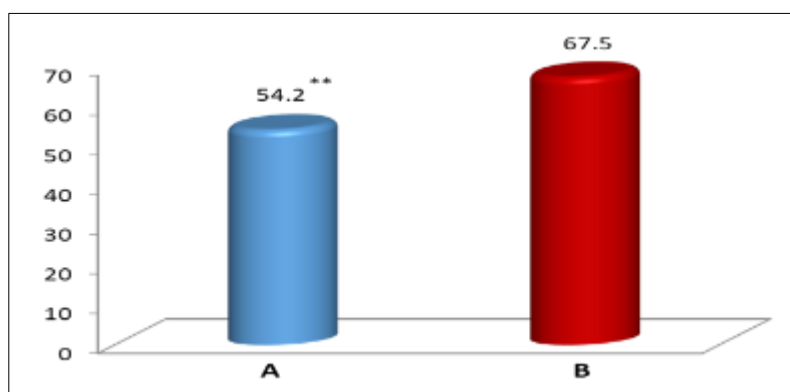
We performed several *in vitro* antioxidant assays to measure the reducing power and radical (DPPH and ABTS) scavenging activity. The study revealed AERGP's dose-dependent radical scavenging effect in both ABTS ( $IC_{50}$  20.0  $\mu\text{g/mL}$ ), and DPPH ( $IC_{50}$  39.0  $\mu\text{g/mL}$ ) experiments.

### 3.3.2. Antiplasmodial assay

AERGP was evaluated against *Plasmodium falciparum* (3D7) and revealed a moderate but effective antiplasmodial activity ( $IC_{50}$  = 47.2  $\mu\text{g/mL}$ ). To our knowledge, such activity of the aqueous extract of rose oil waste from the *R. gallica* Georgian cultivar was not previously described.

### 3.3.3. Carrageenan edema assay

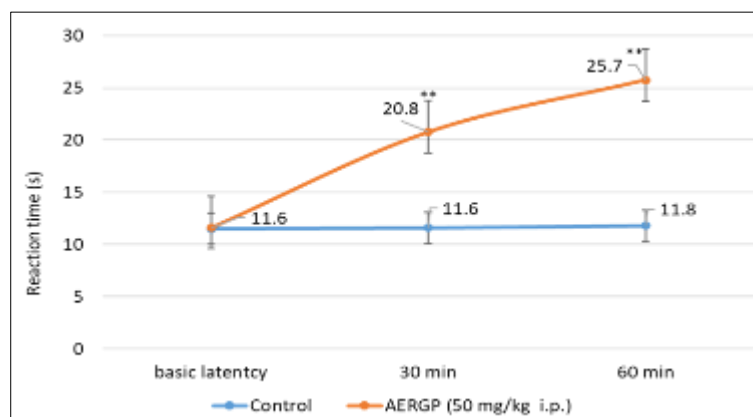
In the experimental group animals, 2 hours after the carrageenan administration, an increase in the thickness of the paw was  $26.0 \pm 7.6 \mu\text{m}$ , which was three times less than in control group animals ( $80.2 \pm 16.4 \mu\text{m}$ ). The efficacy of AERGP was 67.6 % at 2 h ( $p < 0.01$ ), evidencing a pronounced anti-inflammatory activity (Fig.3). Our results correlate with literary data [28],[29],[30], thus confirming the anti-inflammatory potential of aqueous quercetin-containing extracts.



**Figure 3** Anti-inflammatory activity of AERGP. A – difference in paw thickness increase ( $\mu\text{m}$ ) between control and AERGP (50 mg/kg, i.p) treated mice, data represented as mean; B – anti-inflammatory effect in %. \*\* -  $p < 0.01$  vs control

### 3.3.4. Hot plate assay

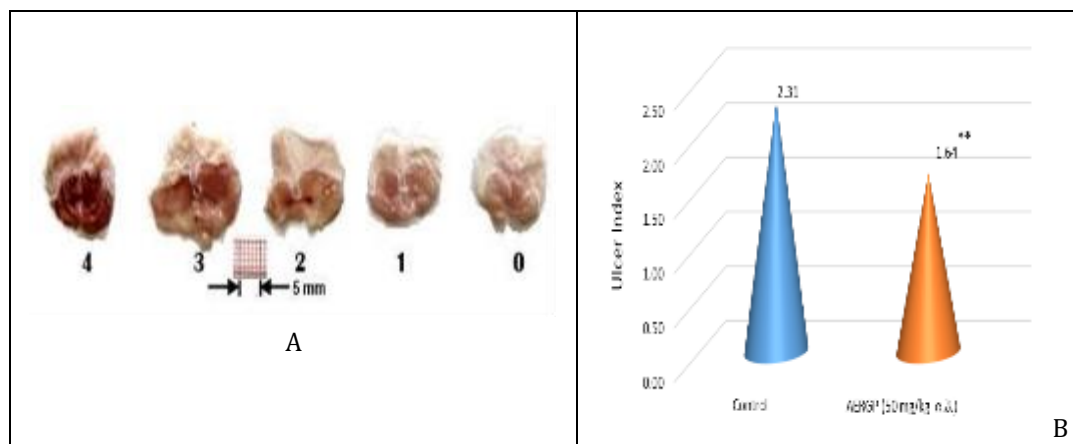
The hot-plate latency in the experimental group was  $11.5 \pm 3.5$  seconds before the administration of 50mg/kg AERGP,  $20.7 \pm 3.7$  and  $25.7 \pm 3.7$  seconds on the 30th and 60th minutes after the injection, respectively; in the control animals the hot-plate latency was  $11.5 \pm 3.5$ , and  $11.7 \pm 2.4$  seconds, respectively. The maximal analgesic effect of the AERGP - 124% ( $p < 0.01$ ), was observed 1 hour after the administration of the extract. Hence, the AERGP exhibits short-term analgesia that confirms data [15],[27],[28] on the analgesic effect of extracts similar in flavonoid composition (Fig.4).



**Figure 4** Analgesic activity of AERGP. Data represented as mean  $\pm$  SEM; Y-axis– reaction time in seconds; X-axis,– measurement time in minutes; \*\* -  $p < 0.01$  vs control

### 3.3.5. Gastroprotective assay

In the ethanol-induced gastric ulcer model in rats, AERGP displayed moderate gastroprotective activity. In control animals, administration of absolute ethanol induced marked gross mucosal lesions, including full length haemorrhagic streaks along the longitudinal axis of the glandular part of stomach and petechial lesions (Fig 5, A.4). In mice given 50 mg/kg i.p. AERGP mainly partial length longitudinal hemorrhagic streaks were observed (Fig 5, A.2). Correspondingly, the UI was significantly reduced in animals pretreated with AERGP 50 mg/kg (UI=1.64±0.84) compared to untreated mice (UI=2.31±0.99, Fig. 4, B). The gastroprotective efficacy of the AERGP was 28.9%.



**Figure 5** A – Ethanol induced ulcer lesions; B – Macroscopic ulcer index (UI) in control and AERGP treated mice. Values represented as mean UI of 6 animals; \*\* -  $p < 0.05$  vs control

## 4. Conclusion

The present study provides data on chemical composition and biological potency of the aqueous extract of rose oil waste from the *R. gallica* 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 quercetin 3-O-galactoside) 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 Pharmacochimistry 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-56-2022).

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