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# Antioxidant Activity of Dry Extract from *Artemisia rupestris* L.

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**ABSTRACT**

The aim of this study was to evaluate antioxidant activity of an extract from *Artemisia rupestris* L. After preparation of a crude extract from *Artemisia rupestris* L., the antioxidant activity of the extract was determined for the first time using $ABTS^{+}$ application method. Trolox was used as positive control. According to the results, we know that one compound of the extract has high antioxidant activity. The compound that shows high antioxidant activity has been determined by HPLC/MS.

**Keywords:** *Artemisia rupestris* L., Antioxidant activity, Extract, HPLC.

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## INTRODUCTION

*Artemisia rupestris* L. grows mainly in Xinjiang Province of China, Kazakhstan, Mongolia, Russia and some European countries (e.g. in the Baltic region and Germany) [1,2]. *Artemisia rupestris* L. is endemic to Kazakh flora and used in Kazakh folk medicine as anticancer, antianaphylaxis and antidote agents [3]. It is a well-known rich source of unique sesquiterpenes such as rupestonic acid which shows anti-virus activity. Investigations of the activity of rupestonic acid against viruses showed that it is highly active against type B flu virus [4,5]. It is also used in traditional Chinese medicine to treat flu [6,7]. *Artemisia rupestris* effectively suppresses inflammatory responses. The mechanism of its anti-inflammatory action is related to its ability to reduce the generation of inflammation mediators [8]. Some research provided guidance for us a novel series of rupestonic acid derivatives valid for the development of new anti-influenza viral drugs [9,10]. As a traditional medicine, *Artemisia rupestris* L. exhibits activities of detoxification, anti-hypersusceptibility, antitumor, antibacterial, antiphlogistic, hemostatic and protecting liver [11, 12]. In Kazakh folk medicine, A. rupestris is prepared as tea and drink to treat some diseases, such as cancers, stomach pain, indigestion, jaundice, flu, urticaria and various types of hepatitis [13]. Crude extract obtained from the tea of A. rupestris by evaporation, is usually used for treatment of skin diseases, e.g., neurodermatitis, poisonous insect bites, and all kinds of skin injuries [14]. In the present study, a crude extract was prepared from *Artemisia rupestris* L., using the traditional extraction method and evaluated for its antioxidant activity for the first time. Antioxidant activity was determined by HPLC, which was previously used for the measurement of antiradical activity profiles of free radical scavengers (called antioxidants) in plant extracts [15].

## MATERIALS AND METHODS

### Chemicals

Gallic, caffeic, chlorogenic, p-coumaric, benzoic, gentisic, cinnamic acids, and ABTS were purchased from Fluka (Buchs, Switzerland), potassium persulphate was from Merck (Darmstadt, Germany), and NaCl, KCl, Na2HPO4, KH2PO4 were from Lachema (Brno, CZ). Acetonitrile of supragradient HPLC grade and water of gradient HPLC grade (Scharlau Chemie S.A., Spain), formic acid, p.a. from Lachema (Brno, CZ) were used. The mobile phase for gradient elution consists of acetonitrile and an aqueous solution of 0.2% formic acid (except when applied to Paulownia tomentosa in which a special mobile phase gradient was used as described in Section 2.3.2). Phosphate buffered saline (PBS) was prepared by Weighing NaCl, KCl, Na2HPO4, KH2PO4 into final concentrations of 137, 2.7, 10.0, 1.76 mmol/l, respectively (pH = 7.4), in water.

### Reagent stability (stock solution)

The active form of ABTS was prepared by mixing equal volumes of aqueous solutions of 14 mM ABTS and 5 mM potassium persulphate. After a period of incubation (overnight), the solution was diluted with water by 80 times. Although the absorbance of the reagent gradually decreased (see Section 3), it should be noted that during a single run, the concentration of the reagent can be considered constant. Moreover, the absolute absorbance of ABTS_+ is not critical for the bleaching reaction until extreme concentrations of analytes are involved (upper LOD, see below).

### Plant material

The aerial parts of *Artemisia rupestris* L. were collected from Altai Mountain, East Kazakhstan region in August 2012. The plant was identified and authenticated by the herbalist at the herbarium of Food and Drug Inspection Center, Xinjiang, China. A voucher specimen (No. 2013S0029) was deposited at the same place.

### Extract preparations

A water extract of *Artemisia rupestris* was made from *Artemisia rupestris* stem, the water was heated 2 h under reflux apparatus at 100°C temperature. This water extract was concentrated in low-pressure evaporator at 40 °C, 35 mbar to a dark gum. The dark gum was Freeze-dried to dry extract. 10 mg of the dry extract was dissolved in 1 ml of methanol.

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**Mobile phase**

**Mobile phase composition**

Linear gradient – 0 min: 10% acetonitrile + 90% formic acid (0.2% aq. solution); 13.0 min: 42.5% acetonitrile + 57.5% formic acid; 13.1 min: 8% acetonitrile + 21% formic acid + 71% methanol; 26 min: 8% acetonitrile + 19.5% formic acid + 72.5% methanol; 26.1 min: 100% acetonitrile; 30 min: 10% acetonitrile + 90% formic acid.

**Mobile phase composition**

The linear elution gradient of acetonitrile was 2.5%/min, starting at 10% of acetonitrile, and ending after 36 min with 100%. Then, the elution with 100% acetonitrile was performed for the next 5 min.

**MS Parameters**

ESI neg. Mode, Scan mode: standard – normal; Nebulizer: 50,0 psi, dry gas: 10,0 l/min. dry temp: 350oC, target mass: 300 m/z; Compound Stability: 100%, trap drive level: 100%, optimize: normal; Trap ICC: yes, target: 30000, max. accu time:300,00 ms, scan: 50-1300 m/z; Averages: 3, rolling averaging: on (2), no of precursors: 2.

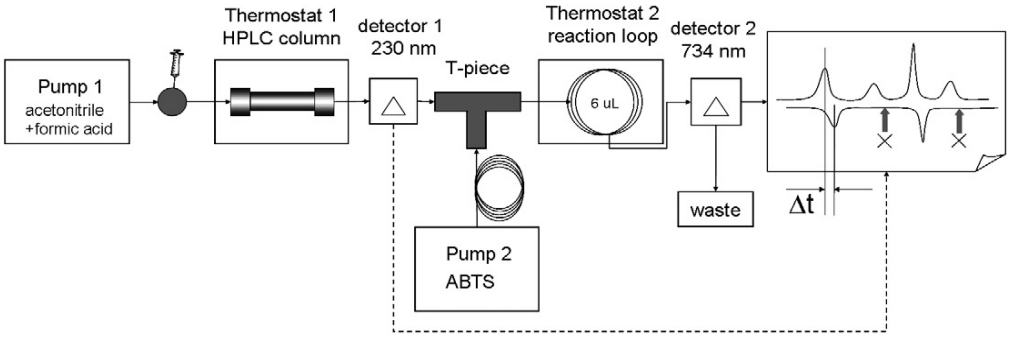
**Columns**

Two RPHPLC columns were used. Column I was an Agilent XDBC18 (5 cm _ 2.1 mm/1.8 lm); column II was a Supelco Supelcosil ABZ+ plus (alkyl-amide reversed phase) 15 cm _ 4.1 mm/3 lm.

**Apparatus**

For photometric measurements, a UV–VIS spectrophotometer HP8453 (Agilent, Germany) was used. For the radical scavenger assay, modules of the HPLC system Agilent 1100 series from Agilent, Germany, were connected according to the scheme in (Figure 1).

**Figure 1: Scheme of the apparatus**




Modules of the apparatus were connected by PEEK tubing (1/160 0 _ 0.13 mm I.D., red stripe). A PEEK 3-way T-piece connects Detector 1 and Thermostat 2 by tubing with a total length of 46 cm, another 20-cm PEEK tubing connects Thermostat 2 with Detector 2. Three to 7 m of the same PEEK tubing after Pump 2 increased back-pressure and worked as a pulse dampener. To ensure a fast course of the post-column reaction, Thermostat 2 had a temperature of 50 °C.

**Method**

For the method, the first step of a run was separation by RPHPLC recorded with a direct absorbance detector, followed by a post-column reaction with a radical form of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS'+). Then, the effect of color bleaching of ABTS'+ was measured on a second absorbance

detector (indirect detection). A comparison of the two signals distinguished the analyzes with antiradical activity [15].

**Calculation method**

A comparative evaluation of the antioxidant activity of the crude extract from *Artemisia rupestris* L., was calculated according to the formula, as indicated by the authors [15].

$$PSP = \frac{A(x)}{Area(total)^*} \times 100\%$$

Where A(x) is the peak area of a peak of interest, Area(total) is the sum of the area of all signals.

## RESULTS AND DISCUSSIONS

**Figure 2: HPLC chromatograms of the extract from *Artemisia rupestris* L.**

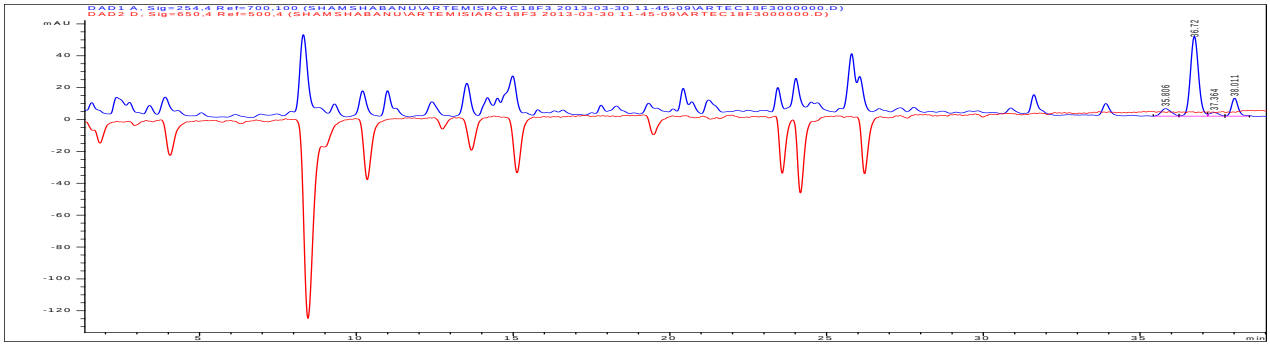



**Figure 3: Densitogram chromatograms of the extract from *Artemisia rupestris* L.**

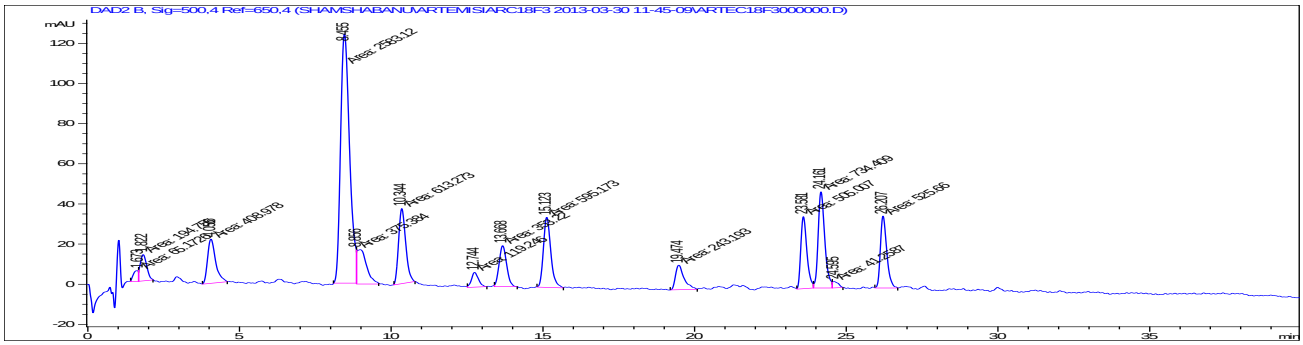



**Table 1: The antioxidant activity of the extract from *Artemisia rupestris* L.**

| Peak № | Area | trolox ekv. [ul/l] | activity [%] |
|---|---|---|---|
| 1 | 65.2 | 15.2 | 0.89 |
| 2 | 194.8 | 45.5 | 2.65 |
| 3 | 409 | 95.6 | 5.56 |
| 4 | 2583.1 | 603.5 | 35.11 |
| 5 | 375.4 | 87.7 | 5.10 |
| 6 | 613.3 | 143.3 | 8.34 |
| 7 | 119.2 | 27.9 | 1.62 |
| 8 | 353.2 | 82.5 | 4.80 |
| 9 | 595.2 | 139.1 | 8.09 |
| 10 | 243.2 | 56.8 | 3.31 |
| 11 | 505 | 118.0 | 6.86 |
| 12 | 734.4 | 171.6 | 9.98 |
| 13 | 41.3 | 9.6 | 0.56 |
| 14 | 525.7 | 122.8 | 7.14 |
| | 7358.0 | 1719.2 | 100 |

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As a result of studying the antioxidant activity of the extract from *Artemisia rupestris* by the ABTS' radical cation using HPLC, the component (№4 peak) of the extract showed high antioxidant activity with a percentage of 35.11%, as indicated in (figure 2, figure 3, table 1).

**Figure 4: HPLC/MS chromatogram of the extract from *Artemisia rupestris* L.**

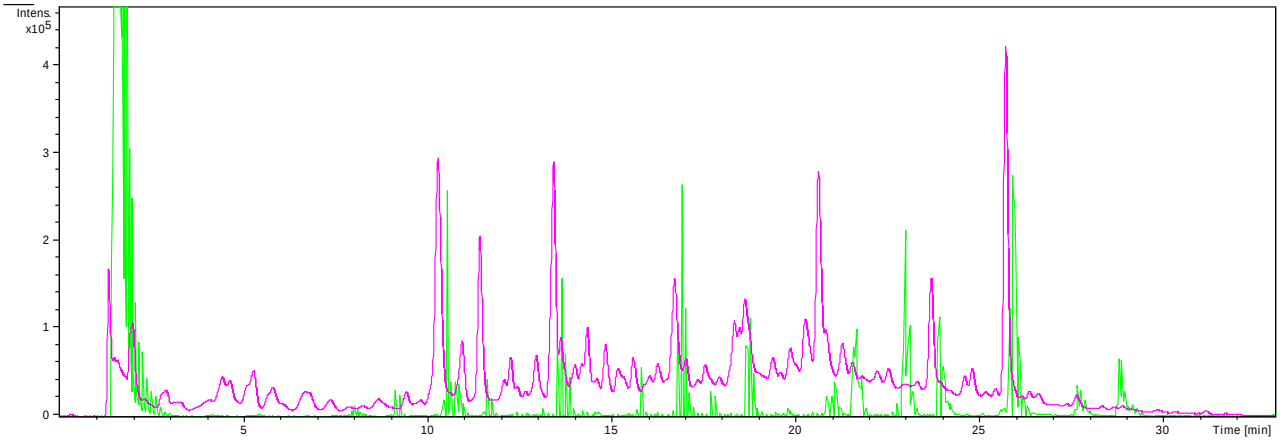



**Figure 5: -MS 10.5 min**

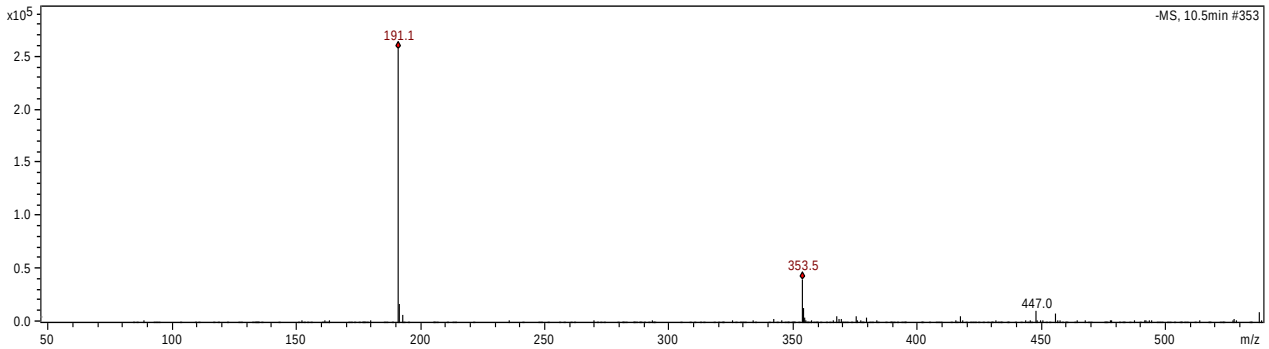



**Figure 6: -MS(191.2).10.6min**

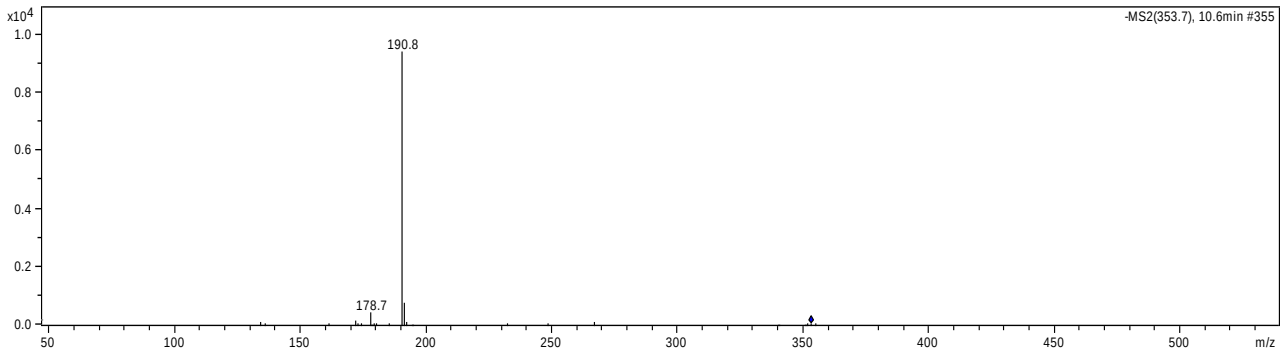
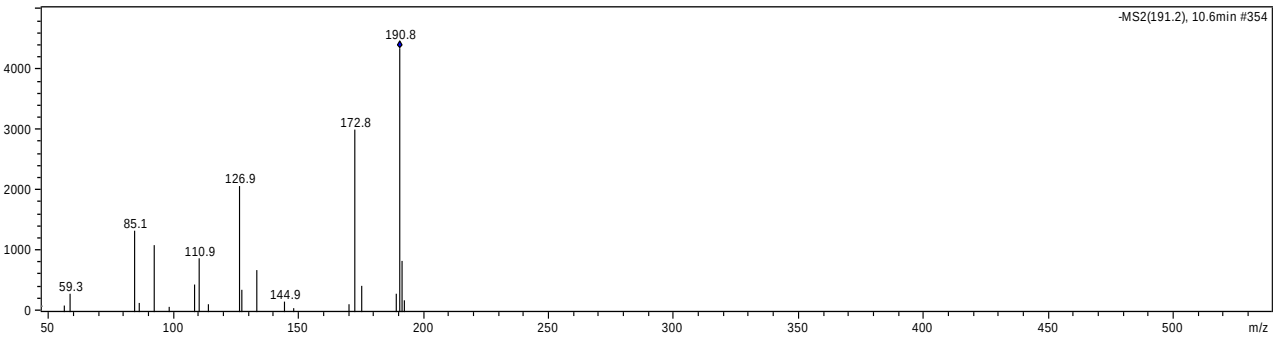



**Figure 7: -MS2(353.7).10.6min**



To determine the compound with high antioxidant activity (№4 peak), the HPLC/MS chromatogram has been used in structure analysis shown in (figure 4). As a result, the mass spectrum of peak №4 showed the [M-H]- ion at m/z 353.5 and 191.1, as indicated in (figure 5). . According to the MS fragmentation patterns, we could say, the second one is the first one's fragment. So there is only one compound which molecular weight is 354.5. $MS^2$ spectra of the ion at m/z 353.5 exhibited a fragment ion with high intensity at m/z 190.8 as well as another fragment ion with low density at m/z 178.7 and was shown in (figure 6), whereas $MS^2$ spectra of the ion at m/z 191.1 exhibited four fragment ions at m/z 190.8, 172.8, 126,9 and 110.9, as indicated in (figure7). By comparing this fragmentation pattern with that of compound described in literature [16], №4 peak was putatively identified as an isomer of caffeoylquinic acid (3-, or 4- or 5- caffeoylquinic acid).

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