



ARISTOLOCHIC ACID I DETERMINATION IN *ARISTOLOCHIA CLEMATITIS* L. RAW MATERIALS BY HPLC METHOD

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Aristolochia clematitidis has long ago been applied in Asian traditional medicine and demonstrates multidirectional pharmacological activity. Contemporary research has confirmed its expressed antimicrobial activity.

Using high performance liquid chromatography (HPLC) we ascertained and determined the content of aristolochic acid I in herb and roots of *Aristolochia clematitidis* collected within the blooming period in Kharkiv and Khmelnytskyi Regions, Ukraine. As aristolochic acid I causes nephrotoxic activity of plants, particularly, *Aristolochia clematitidis*, it is quite mandatory to control its content in raw materials. The content of aristolochic acid I in *Aristolochia clematitidis* herb was $0.11 \pm 0.01\%$, in roots its content was $0.14 \pm 0.01\%$. Thus, issuing from the results of our experiment, we concluded that *Aristolochia clematitidis* raw materials may be treated as feasible for the development of drugs, but only for external application. The results of our study may be used for the development of *Aristolochia clematitidis* raw materials standardization parameters and safe application.

INTRODUCTION

Aristolochia genus is one of the most numerous in *Aristolochiaceae* family and embraces near 500 herbaceous perennial species, many of them are creepers. This species is met in Asia, Africa, North and South America, Australia, especially in tropical Asia¹⁻³. The plants are used in traditional medicine and homeopathy, mainly in Asian countries (China, Bangladesh, etc.)^{1&4}. The plants serve as anti-inflammatory, diuretic means, as well as antimicrobial drug for treatment of urogenital diseases, eczema, fungus cutaneous diseases, snake bites^{1,3,5&6}.

Many researchers experimentally proved antimicrobial activity for *Aristolochia* genus plants: ethanol extract from *Aristolochia*

galeata is active relative to *Staphylococcus aureus*⁷; essential oil from *Aristolochia indica* shows moderately expressed antimicrobial activity, whereas aqueous and ethanol extracts from *Aristolochia clematitidis*, herb and roots collected within the budding and blooming period were studied as regards antimicrobial activity. Antimicrobial activity was most clearly expressed in extracts from this plant collected within the blooming period, especially relative to *Staphylococcus aureus* and *Bacillus subtilis*⁸.

However, *Aristolochia* genus plants possess nephrotoxic and carcinogenic action which may refer to presence of aristolochic acids^{1,9-12}.

Therefore, for safe application of plants containing aristolochic acids, they must be strictly controlled as regards their content.

The most of researches devoted to study of aristolochic acids in plants, particularly, of *Aristolochia* genus, have been devoted to either determination of aristolochic acid I, or separation of mixture of aristolochic acid I and aristolochic acid II^{9,13-15}.

Aristolochic acid I (synonym: aristolochic acid AA-I) is a nitrophenanthrene carboxylic acid, considered to be one of the most active substances in *Aristolochia* genus plants² (Fig. 1).

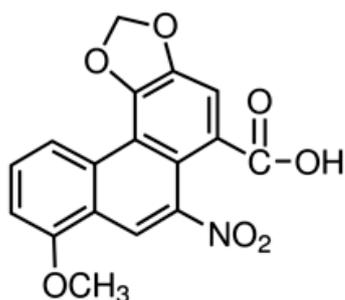


Fig. 1: Structural formula of aristolochic acid I.

In European countries, particularly in Ukraine, *Aristolochia clematitis* grows as a weed. This plant is not included to Pharmacopoeia, its application has certain limitations, but it is promising in some aspects, particularly as a raw material for the development of externally applied drugs.

Aristolochia clematitis raw material requires a thorough study, including determination of aristolochic acid I which is mandatory for creation of safe drugs. Its content in plant must be established and controlled.

Therefore, the present work is devoted to determination of aristolochic acid I in *Aristolochia clematitis* herb and roots.

MATERIALS AND METHODS

Plant Materials

Aristolochia clematitis herb and roots were collected within the blooming phase in Kharkiv and Khmelnytskyi Regions, Ukraine, in May-June 2019/2020.

Plant samples were identified by Professor Iryna Zhuravel, Department of Chemistry of Natural Compounds and Nutritiology, National

University of Pharmacy, Ukraine. The voucher specimen was deposited at National University of Pharmacy (Ukraine) with certain number 1506/2019.

Research methodology and chromatography conditions

As the State Pharmacopoeia of Ukraine (SPU) has been a member of the European Pharmacopoeia (EP) since 2013, the methods specified therein are associated with the EP requirements (except national part). According to both EP and SPU, for raw materials containing aristolochic acids their determination is mandatory.

Thus, for our research we applied SPU 2.0 Method 2.8.21 "Aristolochic acids content in herbal raw material test"^{16&17}.

The chromatographic study of tested herb specimens was performed at a Shimadzu HPLC-system, ser.20 liquid chromatograph equipped with a diode matrix detector under the following conditions:

- Xterra MS C18 column, dimensions: 150 mm x 4,6 mm, particle size 3,5 µm;
- Column temperature 40°C;
- Detector wavelength 390 nm;
- Mobile phase flow rate 0.3 ml/min;
- Introduced sample volume 25 µl.

The mobile phase is shown in table 1.

Table 1: Mobile phase.

Chromatography time, min	Eluent A, ^a %	Eluent B, ^b %
0 – 25	85 → 35	15 → 65
25 – 30	35 → 0	65 → 100
30 – 31	0 → 85	100 → 15

^aEluent A: 0.1% aqueous solution of trifluoroacetic acid.

^bEluent B: 0.1% acetonitrile solution of trifluoroacetic acid.

The components were identified by their retention time and conformity of their UV spectra to standard substance.

The calculations were performed by the equation:

$$X, \% = \frac{A_{pr} \times m_{st} \times V_{pr} \times P \times 100}{A_{st} \times V_{st} \times m_{pr} \times 100} \dots\dots\dots(1)$$

where: A_{pr} – substance peak area in tested solution chromatogram; A_{st} – substance peak area in reference solution chromatogram; m_{st} – mass of substance standard sample, mg; m_{pr} – mass of tested herb sample, mg; V_{pr} – dilution of tested solution, ml; V_{st} – dilution of reference solution, ml; P – activity of standard, %.

Preparation of analyzed solutions

Tested solution: 1 g powdered raw material was placed to a 250 ml dark glass flask, 100 ml solvent mixture (acetonitrile-water 50:50) was added. It was kept on ultrasonic bath for 30 min and filtered through 0,45 μ m filter.

Reference solution: 1 mg standard aristolochic acid I sample (from ChemFaces, China, Catalog No.CFN99505) was dissolved in 5 ml solvent mixture (acetonitrile-water 50:50). 1 ml of obtained solution was transferred to a 10 ml flask and diluted with a solvent mixture up to the mark. The solution was filtered through 0,45 μ m filter.

Statistical analysis

IBM SPSS Statistics for Windows, Version 26.0 was used for statistical analysis of results. Results are expressed as means of three measurements \pm SD. A probability of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSIONS

HPLC chromatogram of standard substance - aristolochic acid I - is shown in figure 2.

HPLC chromatograms of aristolochic acid I determination in *Aristolochia clematitis* herb and roots are given in figures 3 and 4.

Chromatographic parameters for standard substance aristolochic acid I and aristolochic acid I determination in tested raw materials are specified in table 2.

The results of our research proved that aristolochic acid I content in *Aristolochia clematitis* herb (as dry matter) was $0.11 \pm 0.01\%$, whereas its content in roots was $0.14 \pm 0.01\%$.

Thus, aristolochic acid I dominated in subterranean part of *Aristolochia clematitis*.

Chinese scientists studied 31 samples of *Aristolochia fangchi* Wu raw materials collected in various regions of China and found that the content of aristolochic acids acutely varied depending on the vegetation area of a particular plant. Nevertheless, they remarked that in most cases aristolochic acid I content was much higher than that of aristolochic acid II⁹.

US researchers also confirmed this trend in aristolochic acids accumulation: in most tested raw material samples characterized by the presence of these compounds aristolochic acid I dominated⁵.

However, the scientists from Sudan have established that *Aristolochia bracteolata* contained aristolochic acid I to the amount of 12.98 g/kg, whereas the content of aristolochic acid II was 49.03 g/kg¹⁸.

As regards the comparison of our results with those of other researchers it may be concluded that aristolochic acid I content varies in quite wide range, beginning from traces, and this substance usually dominates among other aristolochic acids.

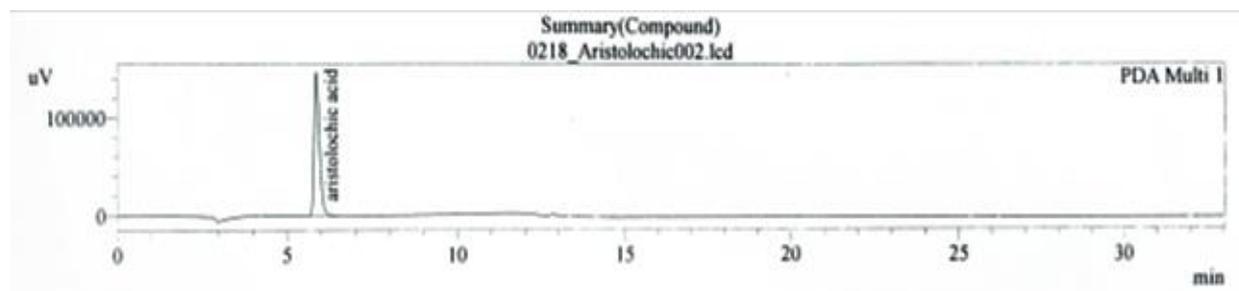


Fig. 2: HPLC chromatogram of standard substance aristolochic acid I.

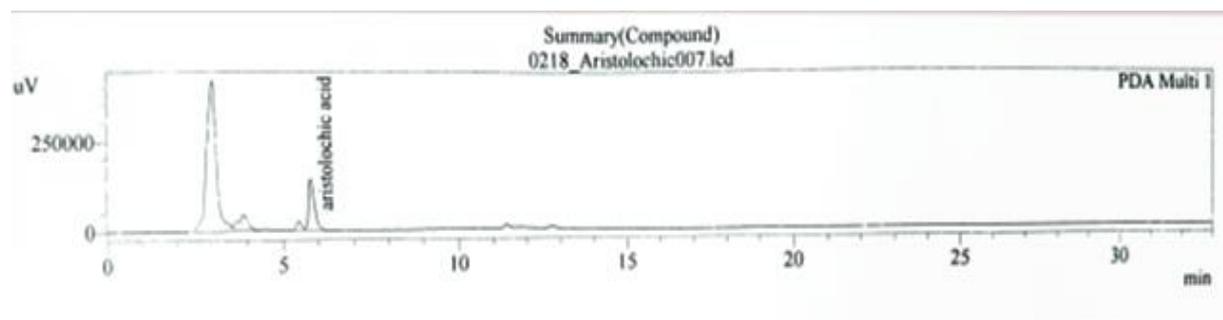


Fig. 3: HPLC chromatogram of aristolochic acid I determination in *Aristolochia clematitis* herb.

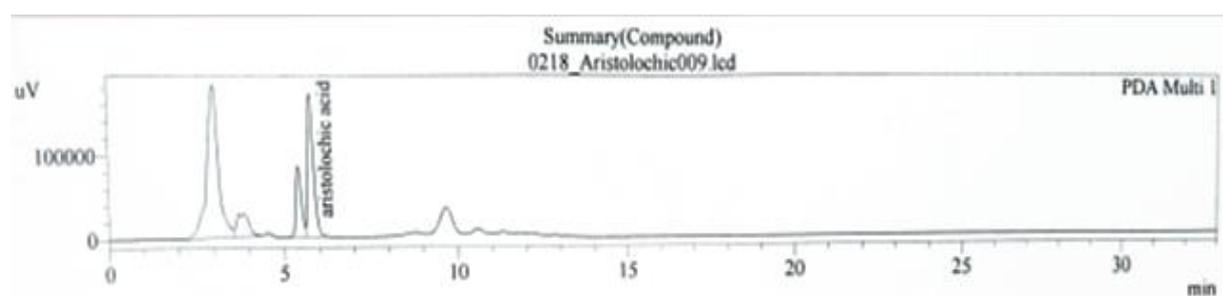


Fig. 4: HPLC chromatogram of aristolochic acid I determination in *Aristolochia clematitis* roots.

Table 2: Chromatographic parameters for determination of standard substance aristolochic acid I and aristolochic acid I determination in tested raw materials.

Title	Retention time	Area	Tailing factor	Theoretical plate	Resolution
Standard substance	5.847 ± 0.022	1616115 ± 4249	1.435 ± 0.008	6231.625 ± 64.228	0.000
<i>Aristolochia clematitis</i> herb	5.810 ± 0.034	1604649 ± 4653	1.443 ± 0.005	5931.127 ± 64.056	1.190 ± 0.003
<i>Aristolochia clematitis</i> roots	5.773 ± 0.027	1934423 ± 4836	1.441 ± 0.007	5784.647 ± 56.689	1.175 ± 0.002

Conclusions

Thus, our comparative chromatographic study established that aristolochic acid I is predominantly accumulated in roots of *Aristolochia clematitis* ($0.14 \pm 0.01\%$). The analysis of our results as well as of the results of other researchers showed that the content of aristolochic acid I varies depending on vegetation area of *Aristolochia* genus plants, particularly, *Aristolochia clematitis*.

Besides, issuing from the previous research, we may conclude that *Aristolochia*

clematitis raw material is feasible for the development of drugs, predominantly for external application.

Control of aristolochic acid I content in *Aristolochia clematitis* herb and roots is mandatory under the development of standardization parameters for this raw material. Therefore, the obtained results may be used for standardization of *Aristolochia clematitis* raw materials as well as for the development of novel drug preparations on the basis of *Aristolochia clematitis* herb and roots.

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The authors declare no conflict of interest, financial or otherwise.

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نشرة العلوم الصيدلانية جامعة أسيوط



تحديد محتوى حمض أرسطولوتشيك ١ في المادة الخام لنبات الأرسطولوكسيا كلماتس باستخدام الكروماتوجرافيا السائلة عالية الأداء (HPLC)

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أوكرانيا

لطالما تم استخدام نبات الأرسطولوكسيا كلماتس في الطب التقليدي الآسيوي وأظهر نشاطًا دوائيًا متعدد الاتجاهات. أكدت الأبحاث المعاصرة نشاطه كمضاد للميكروبات.

باستخدام كروماتوجرافيا سائلة عالية الأداء (HPLC) ، تأكدنا وحددنا محتوى حمض الأرسطولوكيك ١ في عشب وجذور الأرسطولوكسيا كلماتس الذي تم جمعه خلال فترة التفتح في مناطق خاركييف وخميينيتسكي ، أوكرانيا. نظرًا لأن حمض الأرسطولوكيك ١ يسبب نشاطًا سامًا للكلى ، ولا سيما الأرسطولوكسيا كلماتس ، فمن الضروري للغاية التحكم في محتواه في المواد الخام. كان محتوى حمض الأرسطولوكيك الأول في الأرسطولوكسيا كلماتس $0.11 \pm 0.001\%$ ، في الجذور كان محتواه $0.14 \pm 0.001\%$. وهكذا ، انطلاقًا من نتائج دراستنا ، خلصنا إلى أن الأرسطولوكسيا كلماتس كمادة خام يمكن التعامل معها على أنها مجدية لتطوير الأدوية ، ولكن فقط للاستخدام الخارجي. يمكن استخدام نتائج دراستنا في تطوير معايير الرقابة لنبات الأرسطولوكسيا كلماتس والاستخدام الآمن له.