WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 3, 1816-1825. Research Article ISSN 2277– 7105

CAROTENOID PROFILE OF AILANTHUS ALTISSIMA STEM BARK, IN-VITRO ANTIOXIDANT AND ANTINEOPLASTIC ACTIVITIES

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Article Received on
19 Jan 2016,

Revised on 09 Feb 2016,
Accepted on 29 Feb 2016

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ABSTRACT

Ailanthus altissima (Mill.) Swingle (tree of heaven) is a fast-growing invasive species for Bulgaria and Europe and it belongs to the family *Simaroubaceae*. Carotenoid fraction from stem bark of *Ailanthus altissima* was isolated for the first time for this plant species, as well as lutein and lycopene. The main components identified and quantified by HPLC analysis were as follows: β-carotene – 4389.96 ± 120.7µg/g extract, lutein – 2515.11 ± 95.66 µg/g extract and lycopene – 154.77 ± 42.15 µg/g extract. The better antioxidant activity of total carotenoid extract was measured by ABTS assay. Antineoplastic effects of carotenoids on BJ, HepG2 and MDA-MB-231 cell lines were investigated, too. High quantities of pure carotenoids exhibited mainly antiproliferative activity, and MDA-MB-231 cell line was the most sensitive.

KEYWORDS: *Ailanthus altissima*, carotenoids, HPLC, antioxidant activity, antineoplastic.

INTRODUCTION

Ailanthus altissima (Mill.) Swingle (tree of heaven) is a fast-growing tree from the family *Simaroubaceae*, indigenous to Asia.[1] It has been introduced into Europe in 1751, and in the United States in 1784 by a Philadelphian horticulturalist William Hamilton. Chinese immigrants imported this species into Western American states and used it for medicinal purposes.[2] *A. altissima* is one of the three taxa which are considered among the 100 most invasive alien species in Europe.[3]

Tree of heaven is a deciduous tree with smooth, grey bark and a straight bole, reaching up to 20 m in height. It has large, malodorous pinnate leaves, with prominent glands on the back side. Leaflets range from 15 to 41 in number, and total leaf length may reach 1 m. The inflorescence (panicle) is a 10 - 20 cm long, with greenish flowers 6-8-mm in diameter. Fruits are dry schizocarps with wings, one-seeded, and they grow in clusters that may contain hundreds of seeds.[4]

It grows aggressively in harsh environment conditions as abandoned fields or cracked city sidewalks.[5] This invasive success can be attributed mainly to its sexual and asexual reproductive behavior and to the allelopathic compounds found in its roots and leaves.[6]

A. altissima has been used in traditional medicine for treatment of many different diseases as: cough, gastric and intestinal upsets, dysentery, hemorrhoids and as antispasmodic, antiasthmatic, cardiac depressant.[7] The bark of this plant is used as an astringent in traditional Chinese medicine for the treatment of diarrhea, bleeding and tinea.[1]

Previous reports reveal the presence of rich number of compounds in it: proteins,[8] neolignans,[9] coumarins,[10] phenolic acids,[11] flavonoids, tannins,[7] tritrepenoids and cerebrosides,[12,13] sterones,[1] quassinoids,[14,15] volatile oils - a complex mixture that is composed mainly of non-terpenic compounds and sesquiterpene hydrocarbons,[16,17] β-carotene,[18] alkaloids.[19,20]

Being so invasive and widespread, tree of heaven could be regarded as a cheap resource to utilize. The present study was focused on the carotenoid content of *A. altissima* stem bark and its biological effects.

MATERIALS AND METHODS

Plant material

Stem bark of *Ailanthus altissima* (Mill.) Swingle was collected from wild growing trees in *Grancharovo* village, District of Silistra, Bulgaria in April, 2015. Plant material was air-dried in darkness at room temperature. Species identification was carried out at the Department of Botany of University of Plovdiv "*Paisij Hilendareski*", according to Tutin et al. (1976).[21] Voucher specimens of the material were deposited in the Herbarium at the Agriculture University of Plovdiv, Bulgaria (Herbarium SOA).

Extraction of carotenoid fraction from A. altissima stem bark

Fifty grams of grounded bark from plant material were mixed with 50 ml of ethanol and then heated in water bath. After filtration, the solid residue were squeezed and the yellow filtrate was moved into another flask. Then 10 ml of dichloromethane were added to the solid residue, and extracted the mixture for 3-4 min. The extraction with dichloromethane was repeated four times with 10 ml portions each time. All extracts were combined and washed with water into a separatory funnel. The dichloromethane layers were dried over anhydrous sodium sulfate, filtered and evaporated to dryness.[22]

HPLC analysis of carotenoid fraction

The HPLC analysis of carotenoids were performed by a Waters HPLC system, (Milford, MA, USA) equipped with binary pump (Waters 11525), a UV-VIS detector (Waters 2487) and Breeze 3.30 SPA software. Concentration of each compound was calculated on the basis of external standard and was converted to µg compound per g dry weight (µg/g DW). Detailed conditions of HPLC analyses were previously reported.[23]

Evaluation of antioxidant activity

DPPH scavenging assay of carotenoid fraction

The electron donation ability of carotenoids extract was measured by DPPH assay. This assay is based on the bleaching of purple colored methanol solution of DPPH. 0.15 mL extract was mixed with 2.85 mL freshly prepared 0.1 mM methanol solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma). The reaction was performed at 37°C in a dark place. After 15 min, absorbance at 517 nm against methanol was recorded. The antioxidant activity was expressed as % inhibition.[24]

$$I\ \% = \frac{(A_{contol} - A_{sample})}{A_{control}} \times 100$$

Where

$A_{control}$ is the absorbance of the control (containing all reagents except the test compound)

A_{sample} is the absorbance of the test compound.

ABTS antioxidant assay

The ABTS assay was performed as described by Thaipong et al.,[25] with some modifications. Briefly, ABTS radical was generated by mixing aliquot parts of 7.0 mM 2, 2`azinobis (3)-

ethylbenzthiazoline-6-sulfonic acid (ABTS, Sigma) in dd H_2O and 2.45 mM potassium persulfate (Merck) in dd H_2O.

The reaction was performed for 16 h at room temperature in the dark. The generated ABTS radical ($ABTS^{\bullet+}$) was stable for several days. Before analyses, 2.0 ml of $ABTS^{\bullet+}$ solution was diluted with methanol at proportions 1:30 (v/v), so the obtained final absorbance of the working solution was about 1.0 ÷ 1.1 at 734 nm. For the assay, 2.85 ml of $ABTS^{\bullet+}$ solution was mixed with 0.15 ml of obtained extracts. After 15 min, at 37°C in the dark, absorbance was measured at 734 nm against methanol. The antioxidant activity was expressed as % inhibition.

$$I\ \% = \frac{(A_{contol} - A_{sample})}{A_{control}} \times 100$$

Where

$A_{control}$ is the absorbance of the control (containing all reagents except the test compound)

A_{sample} is the absorbance of the test compound.

Cell cultures

The BJ (human skin fibroblast cell line), HepG2 (human hepatocyte carcinoma cell line) and MDA-MB-231 (breast cancer cell line) cells were cultured in Dulbecco Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Austria), 100 U/ml penicillin and 0.1 mg/ml streptomycin (Lonza, Belgium) under 5% CO_2 atmosphere at 37°C. Plastic flasks, supplied by Greiner, Germany, were used to grow the cells. For experiments the cells in exponential phase of growth after treatment with trypsin-EDTA (Flow Lab, Australia) were seeded into 96-well plates (Greiner, Germany) in a concentration 2x104 cells/well.

Cell viability assay

After a 24-hour period of post seeding, the cultivated cells were treated with carotenoid fraction in a concentration range of 2-1000 μg/ml.

Untreated cells were used as controls. Cytotoxicity was measured by colorimetric assay based on tetrazolium salt MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma Chemical Co.). The assay was performed 24 - and 72 - hours after treatment with the

carotenoid fraction extracted from *Ailanthus altissima*. ELISA plate reader (TECAN, Sunrise TM, Grodig/Sazburg, Austria) was used for reading the results. Optical density was determined at a wavelength of 540 nm and a reference wavelength of 620 nm. Cell cytotoxicity determined by MTT assay was expressed as per cent of untreated control.

Statistical Analysis

Results were expressed as arithmetic means ± standard deviation (SD) of the means of three separate experiments (each experiment was done with three parallel probes). The statistical evaluation was performed using parametric unpaired t-test. A difference at $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

The total content of obtained carotenoid extract from *A. altissima* stem bark were 220.10 mg estimated as 0.44% from the plant substances. Lutein, lycopene and β-carotene were identified, except the unidentified compounds that can be subjects of further investigation (Fig. 1). β-carotene was the compound with the highest amount - 4389.96 µg/g (extract), followed by lutein – 2515.12 µg/g (extract) and lycopene – 154.78 µg/g (extract) (Tab. 1).

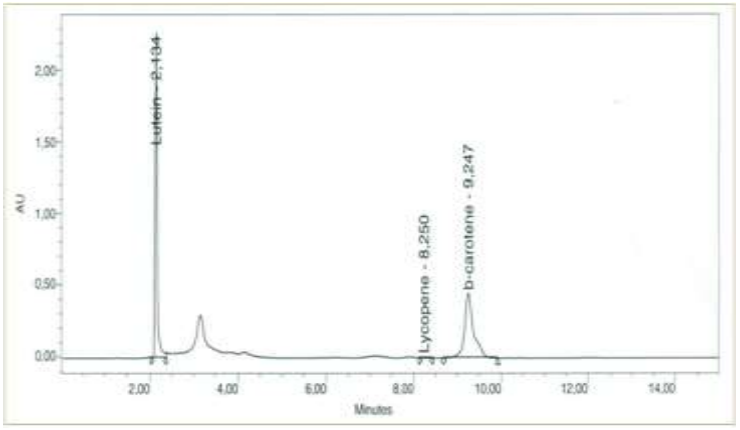


Fig. 1. HPLC chromatogram of carotenoids in *A. altissima* stem bark.

Tab. 1. Content of carotenoids in *A. altissima* stem bark Compounds in carotenoid.

Compounds in carotenoid fraction	***lutein***	***lycopene***	***β-carotene***
Value (µg/g) from extract	2515.12±95.66	154.78±42.15	4389.96±120.7

The presence of carotenoids was mentioned for the first time in the leaves of *Ailanthus altissima*.[17] Carotenoid fraction from stem bark of *A. altissima* was isolated for the first time in the present study, and lutein and lycopene were identified and quantified for the first time in this plant species, too.

There are several described methods to evaluate the *in-vitro* antioxidant capacity of carotenoids and indirect evidence for their *in-vivo* antioxidant activity.[26]

The radical scavenging of carotenoid fraction was investigated by DPPH and ABTS assays. The studied substances showed a good antioxidant capacity, especially regarding the ABTS assay - 112.9±7.0 mMTE/g extr (Tab. 2).

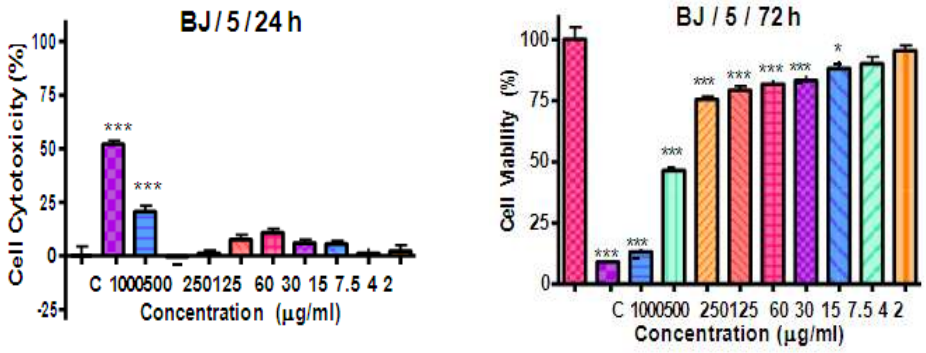
Tab. 2. Antioxidant activity of carotenoid fraction from *A. altissima* stem bark.

Method	***DPPH***	***ABTS***
***Value** (mMTE/g extr)*	69.6±6.6	112.9±7.0

For comparison, the antioxidant activity of *A. altissima* leaves methanolic extracts showed strong concentration-dependent effects with four complementary tests (DPPH, ABTS, 2-deoxyribose and FRAP).[27,28] Other investigations determine ethyl acetate fraction from this plant as superior to all and more potent.[8]

Several recent studies have demonstrated that carotenoid derivatives have negative effect on cell proliferation.[26] In our research, carotenoid fraction from *A. altissima* bark was tested for antineoplastic activity in BJ, HepG2 and MDA-MB-231 cell lines. The carotenoid fraction had concentration-dependent and time-dependent inhibition effects on growth of cell lines (Fig. 2). The most sensitive cell line was MDA-MB-231 with IC50 = 171.1 ± 6.50 µg/ml, followed by non-tumorigenic cell line - BJ with IC50 = 234.2 ± 13.28 µg/ml and second tumor cell line - HepG2 with IC50 = 322.7 ± 6.46 µg/ml (Tab. 3).

A **B**



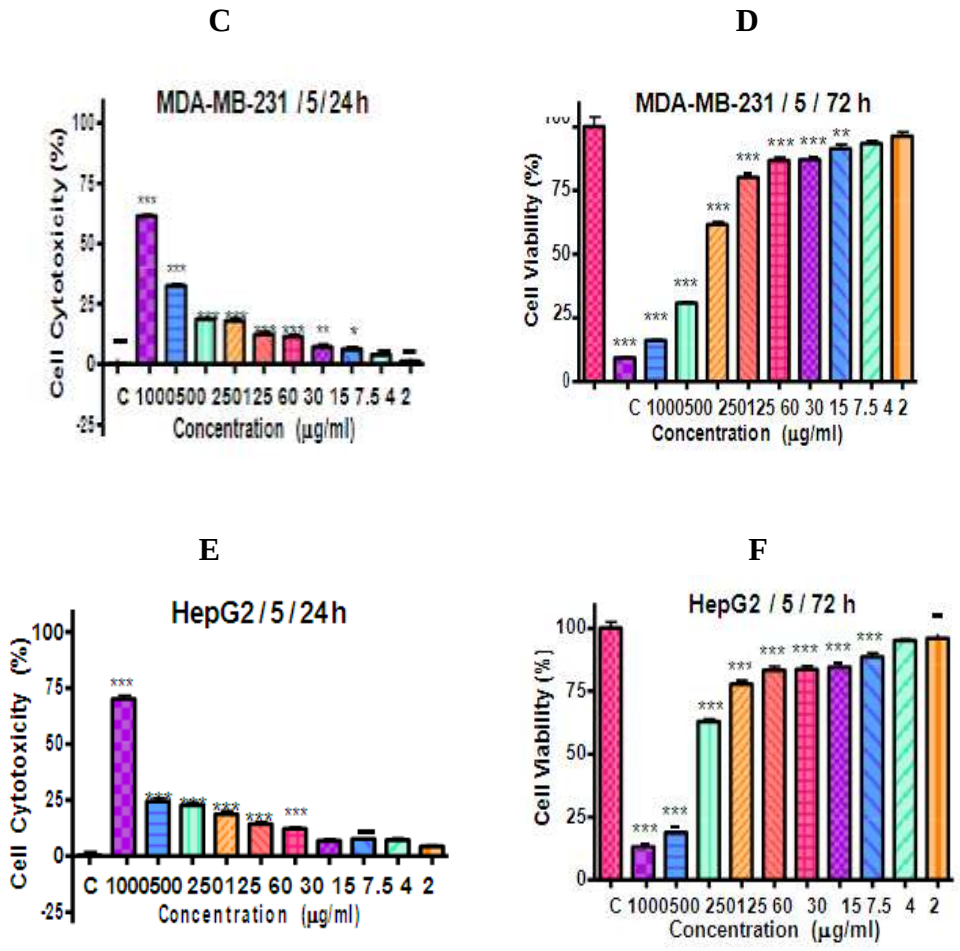


Fig. 2. Antineoplastic activity of carotenoids from *A. altissima* stem bark A B.

Tab. 3. Antineoplastic activity of carotenoids from *A. altissima* stem bark.

	Mean IC_{50} values ± SD (µg/ml)	Mean IC_{50} values ± SD (µg/ml)
Cell lines	**after 24 h**	**after 72 h**
BJ	959.6 ± 31.19	234.2 ± 13.28
MDA-MB-231	805.4 ± 15.20	171.1 ± 6.50
HepG2	780.5 ± 28.93	322.7 ± 6.46

Many epidemiological, clinical studies and those on human cell lines support an inhibitory effect of carotenoids on carcinogenesis. Carotenoids influence aberrant proliferation, apoptosis and mitosis of cells as well.[26]

CONCLUSION

Carotenoid fraction from stem bark of *Ailanthus altissima* was isolated for the first time in the present study. Lutein and lycopene were identified and quantified for the first time for this plant species, too.

The big amounts of obtained pure carotenoids as well as their proven antioxidant and antiproliferative activities give reason to think that the stem bark of tree of heaven could be a valuable source of β-carotene and lutein and a promising base for further studies of the antineoplastic activity.

The results obtained show good availability of the methodology for carotenoid isolation from plant material.

REFERENCES

1. Zhou XJ, Xu M, Li XS, Wang YH, Gao Y, Cai R, Cheng YX. Triterpenoids and Sterones from the Stem Bark of Ailanthus altissima. Bull Korean Chem Soc, 2011; 32(1): 127-30.
2. Masteli J, Jerkovi I. Volatile Constituents from the Leaves of Young and Old Ailanthus altissima (Mill.) Swingle Tree. Croat Chem Acta, 2002; 75(1): 189-97.
3. Kožuharova E, Lebanova H, Getov I, Benbassat N, Kochmarov V. Ailanthus altissima (Mill.) Swingle – a terrible invasive pest in Bulgaria or potential useful medicinal plant? Bothalia J, 2014; 44: 213-30.
4. Al-Snafi AE. The pharmacological importance of Ailanthus altissima - a review. Int J Pharm Rev Res, 2015; 5(2): 121-9.
5. Pan E, Bassuk N. Effects on soil types and compaction on the growth of Ailanthus altissima seedlings. J Environ Hortic, 1986; 4(1): 1-4.
6. Heisey RM. Evidence for allelopathy by tree-of-heaven (Ailanthus altissima). J Chem Ecol. 1990; 16(6): 2039-55.
7. Perry LM. Medicinal plants of East and South East Asia. MIT Press, Cambrigde, Massachusetts, London, England, 1980; 387.
8. Rahman A, Kim EL, Kang SC. Antibacterial and antioxidant properties of Ailanthus altissima swingle leaves extract to reduce foot borne pathogens and spoiling bacteria. J Food Safety, 2009; 29: 499-510.
9. Tamura S, Fukamiya N, Okano M, Koike K. A new quassinoid, ailantinol H from Ailanthus altissima. Nat Prod Res, 2006; 20(12): 1105-9.
10. Dao TT, Tran TL, Kim J, Nguyen PH, Lee EH, Park J, Jang IS, Oh WK. Terpenylated coumarins as SIRT1 activators isolated from Ailanthus altissima. J Nat Prod, 2012; 75(7): 1332-8.
11. Lou KQ, Tang WZ, Wang XJ. Study on chemical constituents from flowers of Ailanthus altissima. Zhong Yao Cai, 2012; 35(10): 1605-7.

12. Hong ZL, Xiong J, Wu SB, Zhu JJ, Hong JL, Zhao Y, Xia G, Hu JF. Tetracyclic triterpenoids and terpenylated coumarins from the bark of Ailanthus altissima (Tree of Heaven). Phytochemistry, 2013; 86: 159-67.
13. Zhao CC, Shao JH, Wang N, Li X, Wang JH. A new cerebroside from fruits of Ailanthus altissima Swingle. Nat Prod Res, 2006; 20(13): 1187-91.
14. Wang Y, Wang WJ, Su C, Zhang DM, Xu LP, He RR, Wang L, Zhang J, Zhang XQ, Ye WC. Cytotoxic quassinoids from Ailanthus altissima. Bioorg Med Chem Lett, 2013; 23(3): 654-7.
15. Yang XL, Yuan YL, Zhang DM, Li F, Ye WC. Shinjulactone O, a new quassinoid from the root bark of Ailanthus altissima. Nat Prod Res, 2014; 28(18): 1432-7.
16. Masteli J, Jerkovi I. Volatile constituents from the leaves of young and old Ailanthus altissima (Mill.) swingle tree. Croat Chem Acta, 2002; 75(1): 189-97.
17. El-Ayeb-Zakhama A, Ben Salem S, Sakka-Rouis L, Flamini G, Ben Jannet H, Harzallah-Skhiri F. Chemical Composition and Phytotoxic Effects of Essential oils obtained from Ailanthus altissima (Mill.) Swingle cultivated in Tunisia. Chem Biodivers, 2014; 11(8): 1216-27.
18. Strain HH. Carotenes from different sources and some properties of σ- and β-carotene, J Biol Chem, 1935; 111: 85-93.
19. Anderson LA, Harris A, Phillipson JD, Production of cytotoxic canthin-6-one alkaloids by Ailanthus altissima plant cell cultures. J Nat Prod, 1983; 46(3): 374-8.
20. Zhang LP, Wang JY, Wang W, Cui YX, Cheng DL. Two new alkaloidal glycosides from the root bark of Ailanthus altissima. J Asian Nat Prod Res, 2007; 9(3-5): 253-9.
21. Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA. (Ed.): Flora Europaea. Vol. 2: Rosaceae to Umbeliiferae, Cambridge (UK); Cambridge University Press, 1968; 230-231.
22. Fieser LF, Williamson KL. Organic Experiments, Seventh Edition, D. C. Heath and Company; Lexington, Massachusetts, USA, 1992; 605-7.
23. Marchev A, Georgiev V, Ivanov I, Badjakov I, Pavlov A. Two-phase temporary immersion system for Agrobacterium rhizogenes genetic transformation of sage (Salvia tomentosa Mill.). Biotechnol Lett, 2011; 33: 1873-8.
24. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical metho d to evaluate antioxidant activity. LWT Food Sci Technol, 1995; 28: 25–30.
25. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava

fruit extracts. J Food Compos Anal, 2006; 19: 669-75.

26. Vahlquist A, Duvic M. Retinoids and carotenoids in dermatology. Informa Healthcare, New York, USA, 2007.
27. Seo YM, Bong HL, Khaled Rashed AS. Antioxidant and cyclooxygenase (COX) inhibitory activities of Ailanthus altissima (Mill.) Swingle leaves. J Biol, 2012; 71(2): 77-84.
28. Ferdaous A, Imed H, Hervé C, Karim H. Phytochemicals, antioxidant, antimicrobial and phytotoxic activities of Ailanthus altissima (Mill.) Swingle leaves. S Afr J Bot, 2013; 87: 164-74.