WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 5, 53-60. Research Article ISSN 2277– 7105

IN-VITRO ANTIOXIDANT AND ANTINEOPLASTIC ACTIVITIES OF CAROTENOIDS FROM FLOWERS OF *KOELREUTERIA PANICULATA*

Iliya Zhelev[1*], Kaloyan Georgiev[1], Ivanka Dimitrova-Dyulgerova[2]

[1]Department of Pharmaceutical technologies, Faculty of Pharmacy, Medical University "Prof. Paraskev Stoyanov"- Varna.

[2]Department of Botany, Faculty of Biology, Plovdiv University"Paisii Hilendarski" – Plovdiv.

Article Received on
02 March 2016,
Revised on 23 March 2016,
Accepted on 11 April 2016
DOI: 10.20959/wjpr20165-6077

***Corresponding Author**
Iliya Zhelev
Department of Pharmaceutical technologies, Faculty of Pharmacy, Medical University "Prof. Paraskev Stoyanov"- Varna.

ABSTRACT

Isolation and quantification by HPLC analysis of carotenoid fraction from flowers of *Koelreuteria paniculata* were done for the first time. β-carotene (2899.95µg/g extract) were the compound with the highest concentration, followed by lycopene (569.87 µg/g extract) and lutein (538.34 µg/g extract). The tested extract showed a good *in-vitro* antioxidant activity by ABTS method (368.86±39.58 mMTE/g extract), whereas antineoplastic potential was week on all tested cell lines.

KEYWORDS: *Koelreuteria paniculata*, flowers, carotenoids, HPLC, antioxidant activity, antineoplastic activity.

INTRODUCTION

Koelreuteria paniculata (Laxm.) is a species that belongs to the family *Sapindaceae*, native to eastern Asia – China and Korea. Common names of this flowering tree include *golden rain* or *China tree, pride of India* and *varnish tree.*[1] It is a popular decorative plant in temperate regions worldwide. In the Bulgarian flora, the plant is introduced and considered invasive species as it inhabits gardens and parks, constantly occupying new habitats.[2]

It is a small to medium-sized deciduous tree, growing to 7 m tall, with a broad, dome-shaped crown. The leaves are pinnate, 15–40 cm long, with 7-15 leaflets (3–8 cm long), with a deeply serrated margin. The flowers are yellow, with four petals, growing in large panicles 20–40 cm long. The fruit is a three-parted inflated bladder like pod 3–6 cm long and 2–4 cm

broad, green ripening orange to pink in autumn, containing several dark brown to black seeds 5–8 mm diameter.[3]

The content of the biologically active substances in different plant parts from *K. paniculata* is mainly represented by phenolic and steroid compounds. The phenolic compounds are derivatives of gallic acid (aglycones and glycosides),[4] flavonoids – mainly aglycones of catechine, isoquercitine, qercitine, quercitrine, kaempferol, hiperoside, as well as their numerous glycosidic forms,[4,5,6,7] and also cyanolipids.[8] The steroid compounds are variety of saponins and sapogenin derivatives of cycloartan,[6] lupinan,[9] oleanan,[10] hederagenin.[11] In our previous investigations carotenoids and antraquinons were found for the first time in this plant species. Amounts of total antraquinons were estimated spectrophotometrically of 0.11%.[12] Studies on pharmacological effects of *K. paniculata* contain its antioxidant activity.[13]

The purpose of this study is to examine the content of carotenoid fraction obtained from inflorescences of *K. paniculata*, growing in Bulgaria, as well as to determine its antioxidant and antineoplastic activity.

MATERIAL AND METHODS

Plant material

Inflorescences of *Koelreuteria paniculata* (Laxm.) was collected in flowering season of July, 2015, from North-East floristic region of Bulgaria. Plant material was air-dried in darkness at room temperature. Species identification was carried out at the Department of Botany of the University of Plovdiv "*Paisij Hilendareski*", according to Tutin et al. (1968); Gramatikov (1992). The voucher specimen was deposited in the Herbarium of the Agricultural University, Plovdiv, Bulgaria (Herbarium SOA – number 060436).

Extraction of carotenoid fraction from A. altissima stembark

Fifty grams of grounded plant material were mixed with 50 ml of ethanol and then heated in water bath. After filtration, the solid residue were squeezed and the filtrate was moved into another flask. Next, 10 ml of dichloromethane were added to the solid residue, and the mixture extracted for 3-4 min. The process of extraction was repeated four times. The combined extracts were washed with water into a separatory funnel. The dichloromethane layers were dried over anhydrous sodium sulfate, filtered and evaporated to dryness. [15]

HPLC analysis of carotenoid fraction

The HPLC analysis of carotenoids was 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 converted to µg compound per g dry weight (µg/g DW). Detailed conditions of HPLC analysis were reported previously. [16]

Evaluation of antioxidant activity

DPPH scavenging assay of carotenoid fraction

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, it was recorded absorbance at 517 nm against methanol. The antioxidant activity was expressed as % inhibition.[17]

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

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., [18] 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 double-distilled water and 2.45 mM potassium persulfate (Merck) in double-distilled water.

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 the extracts obtained. After 15 min, at 37 °C in the dark, absorbance was measured at 734 nm against methanol. The antioxidant activity was expressed as % inhibition.

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

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^{o}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 (FlowLab, Australia) were seeded into 96-well plates (Greiner, Germany) in a concentration $2x10^4$ 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 for 24-and 72-hours after treatment with the carotenoid fraction extracted from *K. panicula*. 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

Carotenoid extract from *K. panicula* flowers was obtained in amount of 1.41 g estimated as 2.82 % from the plant substance. Three carotenoids were identified - β-carotene, lycopene and lutein, except the unidentified compounds that can be subjects of further investigation

(Fig. 1). β-carotene was the compound with the highest amount - 2899.95 µg/g (extract), followed by lycopene – 569.87 µg/g (extract) and lutein –538.34 µg/g (extract) (Tab. 1).

The presence of a new class of natural compounds – carotenoids, was established and estimated for the first time in *K. panicula* in the present study.

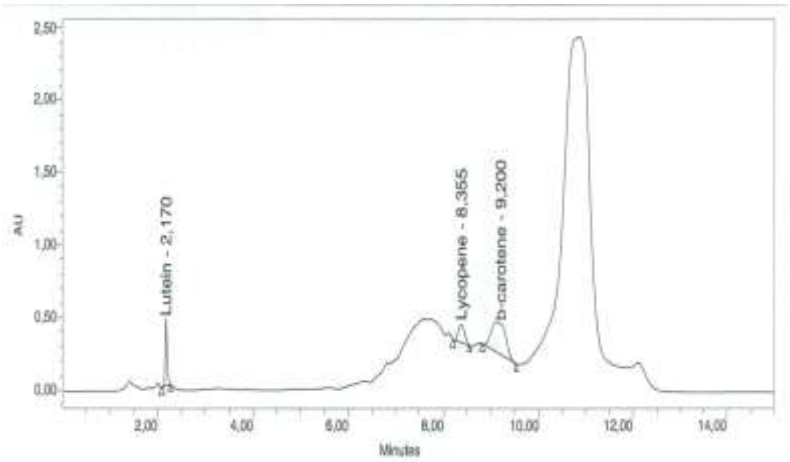

Fig.1. HPLC chromatogram of carotenoids in *K.paniculata* flowers

Tab.1. Content of carotenoids in *K. paniculata* flowers

Compounds in carotenoid fraction	*lutein*	*lycopene*	*β-carotene*
Value** (µg/g) **from extract	538.34±1.41	569.87±3.12	2899.95±5.97

The antioxidant capacity of carotenoids as indirect evidence for their *in-vivo* antioxidant activity was established with several *in-vitro* methods and also evaluated.[19]

DPPH and ABTS assays were used for estimation the radical scavenging of carotenoid fraction in present work. A good antioxidant capacity was established by ABTS assay – 368.86 ± 39.58 mMTE / g (extr). The value by DPPH method was ten time lower 37.06 ± 1.54 mMTE/g (extr) (Tab. 2).

Tab. 2.Antioxidant activity of carotenoid fraction from *K. paniculata* flowers

Method	*DPPH*	*ABTS*
***Value** (mMTE/g extr)*	37.06±1.54	368.86±39.58

In other studies the antioxidant activities of methanolic extract from leaves of *K. paniculata* shows good concentration-dependent scavenging effects on four complementary tests (reducing power, superoxide anion radical scavenging assays, DPPH and ABTS). Simultaneously, fractions from this extract show a DNA protective activity of calf thymus

DNA.[9, 20] Carotenoid derivatives have negative effect on cell proliferation, proved by several studies. Carotenoids influence aberrant proliferation, apoptosis and mitosis of cells as well. [19] In present study, isolated carotenoids from *K. paniculata* flowers were tested on BJ, HepG2 and MDA-MB-231 cell lines for antineoplastic activity. The fraction showed weak inhibition effects on cell lines growing (Fig. 2). The most sensitive cell line was HepG2 with IC_{50} = 459.9 ± 11.63 µg/ml, followed by second tumor cell line MDA-MB-231 with IC_{50} = 522.2 ± 25.91 µg/ml (Tab. 3).

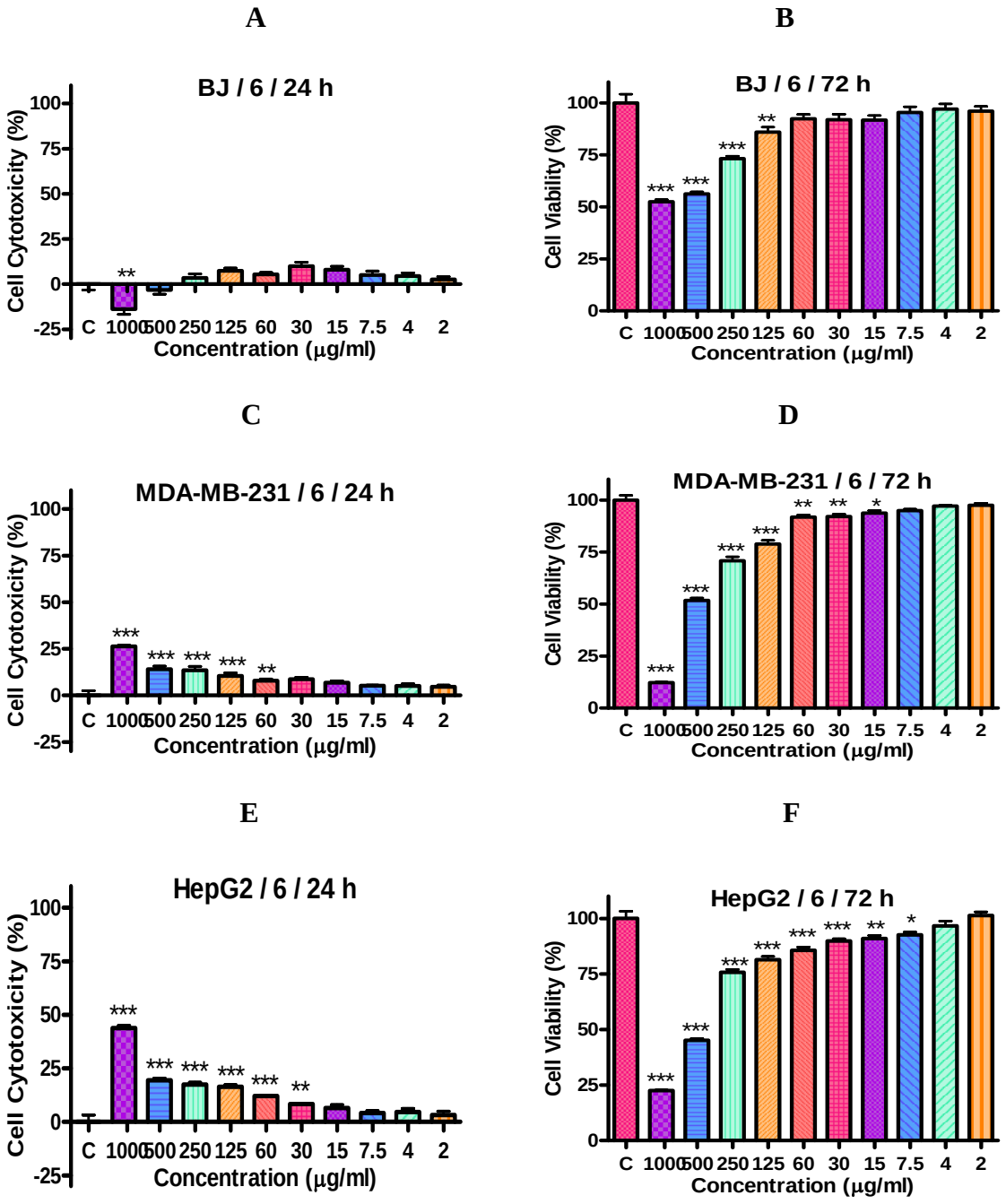

Fig. 2: Antineoplastic activity of carotenoids from *K. paniculata* flowers

Tab. 3.Antineoplastic activity of carotenoids from *K. paniculata* flowers

Cell lines	Mean IC_{50} values ± SD (µg/ml) after 24 h	Mean IC_{50} values ± SD (µg/ml) after 72 h
BJ	>1000	>1000
MDA-MB-231	>1000	522.2 ± 25.91
HepG2	>1000	459.9 ± 11.63

CONCLUSION

Pure carotenoid fraction has been isolated from flowers of *Koelreteria panicula* for the first time. Valuable carotenoid compounds (mainly β-carotene and also lycopene and lutein) were identified for this plant species. Because of the identified high amounts of carotenoids and their proven antioxidant potential, *K. panicula* could be a suitable source of β-carotene, as well as being widely distributed in Bulgaria and worldwide.

REFERENCES

1. http://www.britannica.com/plant/goldenrain-tree
2. Vakarelov I. Decorative dendrology (Bg), Sofia; Zemizdat., 1991; 239-240.
3. Gramatikov D. Key to the trees and shrubs in Bulgaria (Bg), Plovdiv; InetlSis., 1992; 191-192.
4. Lin WH, Deng ZW, Lei HM, Fu HZH, Li J. Polyphenolic compounds from the leaves of *Koelreuteria paniculata* Laxm. J Asian Nat Prod Res, 2002; 4(4): 287–95.
5. Qu QH, Zhang L, Bao H, Zhang JH, You XJ, Wang JX. Chemical constituents of flavonoids from flowers of *Koelreuteria paniculata*. J Chinese Med Mat, 2011; 34(11): 1716-9.
6. Sutiashvili MG, Alaniya MD, Mshvildadze VD, Skhirtladze AV, Pichette A, Lavoie S. Flavonoids and cycloartane glycosides from seeds of *Koelreuteria paniculata*. Chem Nat Compd, 2013; 49(2): 395-7.
7. Xiao FY, Hai ML, Hong ZF, Wen HL. Study on the flavonoids from the seeds of *Koelreuteria paniculata* Laxm. Acta Pharm Sin, 2000; 35(3): 208-11.
8. Mikolajczak KL, Smith CR, Tjarks LW. Cyanolipids of *Koelreuteria paniculata* Laxm. Seed Oil Lipids, 1970; 5(8): 672-7.
9. Lei H, Gong W, Bi W, Zhang J, Lin W. Study on the chemical constitutents from the barks of *Koelreuteria paniculata*. Acta Pharm Sin. 2004; 39(4): 253-4.
10. Lei HM, Li Q, Bi W, Bai D, Lin WH. A new saponin from *Koelreuteria paniculata*. Acta Pharm Sin, 2007; 42(2): 171-3.

11. Chirva VY, Kintya PK, Sosnovskii VA. Triterpene glycoside of *Koelreuteria paniculata*. Chem Nat Comp, 1970; 6(3): 328-30.
12. Zhelev I, Ivanov S, Cherenkova A, Abdulkhakova A, Dimitrova-Dyulgerova I. Phytochemical Study of Flowers from *Koelreuteria paniculata* Laxm growing in Bulgaria. Varna Medical Forum, 2014; 3(4): 312-6.
13. Kumar M, Chandel M, Kumar S, Kaur S. Protective effects of *Koelreuteria paniculata* Laxm on oxidative stressand hydrogen peroxide-induced DNA damage. Phytopharmacol, 2011; 1(5): 177-89.
14. Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds.). Flora Europaea. Vol. 2: Rosaceae to Umbeliiferae, Cambridge (UK); Cambridge University Press., 1968; 230-231.
15. Fieser LF, Williamson KL. Organic Experiments, 7-th ed., Lexington, Massachusetts, USA; D. C. Heath and Company., 1992; 605-607.
16. 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.
17. Brand-Williams W, Cuvelier ME, Berset C. Useof a free radical method to evaluate antioxidant activity. LWT Food SciTechnol, 1995; 28: 25–30.
18. 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.
19. Vahlquist A, Duvic M. Retinoids and carotenoids in dermatology. New York, USA; Informa Healthcare., 2007.
20. Kumar M, Chandel M, Kumar S, Kaur S. Studies on the Antioxidant/Genoprotective Activity of Extracts of *Koelreuteria paniculata* Laxm. Am J Biomed Sci, 2012; doi: 10.5099/aj120200132.