

CYTOTOXIC, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF *AMPELOPSIS BREVIPEDUNCULATA* AND *PARTHENOCISSUS TRICUSPIDATA* (VITACEAE)

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Abstract — Cyclohexane and methanol extracts of leaves and inflorescences of *Ampelopsis brevipedunculata* and *Parthenocissus tricuspidata* are shown to exert significant cytotoxic action on both estrogen-dependent (MDA-MB-361) and estrogen-nondependent (MDA-MB-453) breast cancer cell lines. Methanol extracts of *P. tricuspidata* exhibited higher cytotoxicity for the MDA-MB-453 cell line (inflorescence: $IC_{50} = 111.45 \pm 2.56 \mu\text{g/ml}$; leaves: $IC_{50} = 56.76 \pm 7.11 \mu\text{g/ml}$) than for MDA-MB-361. Cyclohexane extracts of *A. brevipedunculata* leaves exhibited high cytotoxicity against MDA-MB-453 ($IC_{50} = 78.32 \pm 0.1 \mu\text{g/ml}$) and the estrogen-dependent MDA-MB-361 cell line ($IC_{50} = 97.40 \pm 2.61 \mu\text{g/ml}$). The highest DPPH-scavenging ability was exhibited by methanol extracts of *P. tricuspidata* inflorescences, with $IC_{50} = 7.55 \pm 0.07 \mu\text{g/ml}$. The tested extracts possessed weak antimicrobial activity.

Key words: Cytotoxicity, antioxidant activity, antimicrobial activity, *Ampelopsis brevipedunculata*, *Parthenocissus tricuspidata*, Vitaceae

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INTRODUCTION

Plants of the family Vitaceae belong to the specific life form of climbing plants. Based on their characteristic adaptive strategy regarding water supply, use of light energy, and some other ecological factors, their structural and functional adaptations define them as a specific ecological type (Metcalfe and Chalk, 1950).

The cytotoxicity, antioxidative properties, and antimicrobial activities of two species of climbing plants, *Ampelopsis brevipedunculata* (Maxim.) Trautv. and *Parthenocissus tricuspidata* (Sieb. & Zucc.) Planch. (Vitaceae), were studied using *in vitro* tests. Both species originate from E and SE Eurasia. Introduced to Serbia as horticultural species, they acclimated successfully and today are in use as garden plants. Ethanol extracts of *A. brevi-*

pedunculata berries have traditionally been used to treat liver disease, and the value of such treatment was confirmed by *in vitro* and *in vivo* tests. The proposed mechanism of action was through inhibition of reactive oxygen species generation from primary injured hepatocytes in the presence of Fe(II), as well as through induction of cellular stress gene expression (Yabe et al., 1997; Yabe and Matsui, 1998; Wu et al., 2004). Different classes of compounds — ionone, phenylpropanoid glycosides, and hydroquinone glucosides, as well oligostilbenes (ampelopsins) and triterpenes could be responsible for the pharmacological activity of *A. brevipedunculata* (Oshima and Ueno, 1993; Inada et al., 1991; Xu et al., 1995). Also, *A. brevipedunculata* extracts inhibited the formation of collagen fibers by rat hepatic M cells (Yabe and Matsui, 1997) and showed antimutagenic action (Lee and Lin, 1988). Stilbene derivatives have been well studied in

the stem, wood, and leaves of *P. tricuspida*. Isolated stilbenes possessed strong antioxidant properties, as well antiplasmodial activity *in vitro* (Kim et al., 2005; Son et al., 2007; Tanaka et al., 1998). There are no data on antimicrobial activity of these two Vitaceae species, except for antiviral activity of *A. brevipedunculata* (Sun et al., 1986).

In view of the well-known antioxidant, anticancer, and estrogenic activities possessed by stilbene derivatives (Murias et al., 2005) and by flavonoids and caffeic acid derivatives (Galati and O'Brien, 2004; Saleem et al., 2004), the aim of present study was to investigate potential antioxidant, cytotoxic, and antimicrobial activities of extracts of leaves and inflorescences of *P. tricuspida* and *A. brevipedunculata*.

MATERIAL AND METHODS

Plant material

Leaves and inflorescences of *Ampelopsis brevipedunculata* and *Parthenocissus tricuspida* were collected in June of 2007 in the Botanical Garden in Belgrade (Serbia). Voucher specimens are preserved in the herbarium of the Institute of Botany, Botanical Garden, University of Belgrade (BEOU 16253; BEOU 16252). The plant material was then dried and ground into powder.

Extraction

Dried leaves (100 g), inflorescences (50 g), and fruits (10 g) of *P. tricuspida* and *A. brevipedunculata* were macerated with cyclohexane (1: 10; 2 x two days), followed by methanol (70%, v/v) extraction using the same procedure. The solvents were evaporated under reduced pressure at 40°C. The composition of all extracts was monitored by TLC and HPLC. Different *in vitro* tests were used to assay the cytotoxicity and antimicrobial activities of cyclohexane and methanol extracts and the antioxidant activity of methanol extracts.

HPLC analysis

Separation by HPLC was performed using an Agilent 1100 Series system equipped with a G-1312A binary pump, a G-1328B injector (20- μ L loop), and a

G1315B DAD detector. The column used was of the ZORBAX Eclipse XDB-C18 type (4.6 \times 250 nm, 5 μ m) and operated at a temperature of 25°C. Gradient elution was performed with solvents A (H₂O and H₃PO₄, pH=2.8) and B (solvent A: acetonitrile) as follows: 10-15% B (5 min), 15-20% B (10 min), 20-30% B (20 min), 30-40% B (10 min), 40-50% B (5 min), 50-70% B (5 min), and 70-10% B (5 min) at a flow rate of 0.8 mL/min. The injection volume was 20 μ L. The compounds present were determined on the basis of their retention times and UV spectra, and by direct comparison with standards when available.

DPPH test

The DPPH radical scavenging activity of methanol extracts was measured according to the modified method described previously by Kundaković et al. (2006). Dry plant material was dissolved in methanol in a concentration 1 mg/ml. Dose-response curves were constructed and IC₅₀ values were calculated. All measurements were performed in triplicate.

TBA test

The inhibitory effect of MeOH extracts on lipid peroxidation (LP) in liposomes was determined using the modified spectrophotometric method described by Kukić et al. (2006). Different quantities (10-250 μ l) of 1% extract solution were used, and LP was induced with FeSO₄ and ascorbic acid. The absorbances of supernatants were measured at 533 nm.

Cytotoxicity in vitro

Cytotoxicity was tested on the MDA-MB-361 (estrogen-dependent) and MDA-MB-453 (estrogen-non-dependent) breast cancer cell lines and on healthy peripheral blood mononuclear cells (PBMC). Ellagic acid and cis-DDR were used as standard substances.

Treatment of cell lines

Stock solutions (50 mg/ml) of extracts made in dimethylsulfoxide (DMSO) were dissolved in media corresponding to the required working concentrations. Neoplastic MDA-MB-361 cells (7000 cells per well) and neoplastic MDA-MB-453 cells (3000 cells

per well) were seeded into 96-well microtiter plates, and five different doubly diluted concentrations of investigated extracts or compounds were added to the wells 24 h later (after cell adherence). The nutrient medium was RPMI 1640 medium, supplemented with L-glutamine (3mM), streptomycin (100 lg/ml), and penicillin (100 IU/ml), 10% heat inactivated (56 °C) fetal bovine serum (FBS), and 25 mM Hepes. The reaction of the medium was adjusted to pH 7.2 with bicarbonate solution.

Determination of cell survival

Cell survival was determined indirectly by measuring total cellular protein by the Kenacid Blue R (KBR) dye-binding method (Clothier, 1995). Briefly, after 72 h of continuous extract action, the medium was discarded and target cells were washed twice with warm (37 °C) phosphate-buffered saline (PBS). Target cells were then fixed for 20 min with 150 µl of a mixture of methanol and acetic acid (3:1), stained for 2–3 h with 0.04% Coomassie Brilliant Blue R-250 in 25% ethanol and 12% glacial acetic acid, and washed, after which bound dye was dissolved in desorbing solution (1 M potassium acetate, 70% ethanol). Absorbance (A) at 570 nm was measured 2 h later. To get cell survival (%), absorbance of samples with cells grown in the presence of various concentrations of the investigated agent was divided by the control optical density (the A of control cells grown only in nutrient medium) and multiplied by 100. The value of A of the blank was always subtracted from A of the corresponding sample with target cells. The IC₅₀ concentration was defined as the concentration of an agent inhibiting cell survival by 50% compared to a vehicle-treated control. All experiments were done in triplicate.

Antimicrobial activity

Two different methods were used to determine antimicrobial activities: the agar-diffusion method and broth microdilution assay. The tested Gram-positive and Gram-negative bacteria and fungi were *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 10240), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633BB), *Bacillus cereus*

(ATCC11778), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (NCIMB 9111), and *Candida albicans* (ATCC 10259 and 24433).

The complete procedure for the agar-diffusion method was described by A car and Goldstein (1996). The investigated extracts (100 mg/ml) were dissolved in methanol (50%, v/v) or DMSO and then poured into agar or diluted to the highest concentration. The results of agar diffusion assays were evaluated by measuring the inhibition zone (in mm) after incubation. Each assay in this experiment was repeated twice. Ampicillin (10 µg/tbl), amikacin (10 µg/tbl), and nystatin (100 U/tbl) served as positive controls.

Broth microdilution assay was used to determine the minimal inhibitory concentration and minimal bactericidal or fungicidal concentration (MBC) according to C a n d a n et al. (2003).

The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate visible growth. The MBC is defined as the lowest concentration of the extract at which inoculated microorganisms were completely killed. All determinations were performed in duplicate, and two positive growth controls were included.

RESULTS

HPLC analysis

Phytochemical analysis using the HPLC method showed the presence of flavonoids and phenolic acids in aerial parts of the investigated plants. The identified compounds of *A. brevipedunculata* were caffeic acid, ellagic acid, quercitrin, and luteolin-7-O-glucoside in methanol extracts of inflorescences; and kempferol, quercetin, rutin, and luteolin-7-O-glucoside in methanol extracts of leaves. The presence of quercetin-3-O-glucoside, caffeic acid, and luteolin-7-O-glucoside was confirmed in methanol extracts of inflorescences of *P. tricuspidata*, while quercetin-3-O-glucoside as well was detected in methanol extracts of its leaves. The stilbene derivative piceatannol was identified only in leaf extracts of *P. tricuspidata*. These compounds were previously recorded in both plants. The major compounds in

Table 1. Cytotoxicity, LP inhibition, and DPPH radical-scavenging activity of *Ampelopsis brevipedunculata* and *Parthenocissus tricuspidata* extracts (IC₅₀ µg/ml ± SD).

	Cytotoxicity				LP	DPPH
	Cyclohexane extracts		Methanol extracts			
	MDA-MB-361	MDA-MB-453	MDA-MB-361	MDA-MB-453		
<i>A. brevipedunculata</i>						
Inflorescences	117.80 ± 7.16	188.50 ± 2.59	> 500	149.44 ± 11.28	33 ± 1	25.67 ± 2.52
Leaves	97.40 ± 2.61	78.32 ± 0.1	> 500	356.40 ± 13.29	-	30.33 ± 0.58
Fruits	-	-	-	-	-	37.25 ± 0.45
<i>P. tricuspidata</i>						
Inflorescences	167.20 ± 8.52	150.08 ± 6	180 ± 4.02	111.45 ± 2.56	-	7.55 ± 0.07
Leaves	118.34 ± 0.54	209.06 ± 6.27	124.24 ± 11.91	56.76 ± 7.11	960 ± 51	12.87 ± 1.42
Fruits	-	-	-	-	-	27.93 ± 0.12
Ellagic acid	190.35 ± 5.47	74.53 ± 3.23				
Cis-DDR	17.35 ± 0.27	3.96 ± 0.45				

root extracts of *A. brevipedunculata* were oligostilbenes, while flavonoids and ionone derivatives were isolated from leaves (Oshima and Ueno, 1993; Inada et al., 1991; Xu et al., 1995). Caffeic acid esters with antioxidant activity and stilbene derivatives were isolated from the leaves of *P. tricuspidata* (Kim et al., 2005; Saleem et al., 2004; Tanaka et al., 1998).

Antioxidant and radical scavenging activity

Methanol extracts of leaves and inflorescences of *P. tricuspidata* and *A. brevipedunculata* possessed dose-dependent DPPH radical scavenging activity (Table 1). The highest effect was exhibited by methanol extracts of *P. tricuspidata* inflorescences, with IC₅₀=7.55±0.07 µg/ml. An inhibitory effect on LP was exerted by methanol extracts of inflorescences of *A. brevipedunculata* (IC₅₀=0.033±0.001 mg/ml) and leaves of *P. tricuspidata* (IC₅₀=0.96±0.05 mg/ml). Leaf extracts of *A. brevipedunculata* did not attain IC₅₀ (44.4%). Methanol extracts of fruits of neither species had any inhibitory effects on LP.

Cytotoxicity

With the exception of methanol extracts of *A. brevipedunculata*, the tested cyclohexane and methanol extracts showed significant cytotoxic activity on both estrogen-dependent (MDA-MB-361) and estrogen-

nondependent (MDA-MB-453) breast cancer cell lines (Table 1). Methanol extracts of *P. tricuspidata* exerted stronger cytotoxic action on MDA-MB-453 cell lines — with IC₅₀=111.45 ± 2.56 µg/ml for inflorescence extracts and IC₅₀= 56.76 ± 7.11 µg/ml for leaf extracts — than on MDA-MB-361. Cyclohexane extracts of *A. brevipedunculata* leaves exhibited high cytotoxicity against MDA-MB-453, with IC₅₀=78.32 ± 0.1 µg/ml, but also against the estrogen-dependent MDA-MB-361 cell line (IC₅₀=97.40 ± 2.61 µg/ml).

Antimicrobial activity

The given plant extracts manifested weak antimicrobial activity against the tested bacteria and fungi, with the exceptions of cyclohexane extracts of inflorescences and methanol extracts of inflorescences and leaves of *P. tricuspidata*, which showed moderate antimicrobial activity against *C. albicans* and *Micrococcus flavus*, with MIC 3.25 mg/ml. Antiviral activity has been reported for *A. brevipedunculata* (Sun et al., 1986), but there are no data on antibacterial or antifungal activity of the two studied plants.

DISCUSSION

Vitaceous plants are known as a good source of stilbenoids, which are potent chemopreventive agents (Jang et al., 1997). Anticancer activity is usually

connected with antioxidant activity (Murias et al., 2005) because reactive oxygen species damage not only lipids and proteins, but also DNA and RNA in living cells. The mechanism of anticancer and antioxidant activities of resveratrol and other hydroxylated analogs has been studied in detail (Murias et al., 2005), as has that of their estrogenic and antiestrogenic activities (Sano et al., 2006). Piceatannol, a resveratrol metabolite, could be a more effective natural product in cancer treatment because of higher efficacy in inducing apoptosis in many cancer cells (Clement et al., 1998; Potter et al., 2002). Tan et al. (2004) showed *Ampelopsis cantoniensis* crude extract possesses dose-dependent cytotoxic and apoptotic activity in relation to human promyelocytic leukemia HL-60 cells. A compound isolated from *A. japonica*, momordin I, induced apoptosis in HL-60 cells (Kim et al., 2002).

Wu et al. (2004) showed that methanol extracts of stems and roots of *A. brevipedunculata* possess strong antioxidant activity against linoleic acid peroxidation and plasmid DNA oxidation, as well as hydroxyl- and DPPH-scavenging activity. Stilbene derivatives from stems of *P. tricuspidata*, especially piceatannol (Kim et al., 2005), and caffeic acid derivatives from its leaves (Saleem et al., 2004) were found to be potent in inhibition of LP in rat liver homogenate and in DPPH and superoxide anion scavenging (DPPH: $IC_{50}=4.56-14.17 \mu\text{g/ml}$; O_2^- : $IC_{50}=0.58-7.39 \mu\text{g/ml}$).

Our results are in accordance with previously published data because the presence of piceatannol, flavonoids, and phenolic acids was confirmed in methanol extracts of *P. tricuspidata*, while ellagic acid, phenolic acids, and flavonoids were recorded in methanol extracts of *A. brevipedunculata*. Those compounds are well-known antioxidants (Gerhäuser et al., 2003), and their presence could explain the strong inhibition of LP and DPPH radical-scavenging activity. Also, since natural polyphenols exert anticancer action by scavenging free reactive oxygen species (Murias et al., 2005), significant cytotoxicity of the tested methanol extracts could be connected with their presence. Piceatannol, a very potent antitumor agent (Potter et al., 2002), was detected only in methanol extracts of *P. tricuspi-*

data, with very high cytotoxicity against the MDA-MB-453 breast cancer cell line.

Malignant cells from both estrogen receptor-positive (ER+) and estrogen receptor-negative (ER-) cell lines were sensitive to hexanol extracts of *A. brevipedunculata* and *P. tricuspidata*, and to methanol extracts of *P. tricuspidata*, indicating that some compounds whose antiproliferative action is ER-dependent are present in the mentioned extracts. The absence of antiproliferative action of methanol extracts of *A. brevipedunculata* on ER receptor-positive malignant MDA-MB 361 cells suggests that estrogen receptor-dependent antiproliferative activity was lost in these extracts. Ellagic acid, present in methanol extracts of *A. brevipedunculata* inflorescences, exhibited high cytotoxicity for nonestrogen-dependent tumor cells, with $IC_{50}=74.53 \pm 3.23 \mu\text{g/ml}$, and could contribute to the antiproliferative action of those extracts.

The cytotoxicity of cyclohexane extracts, especially those of *P. tricuspidata* leaves and *A. brevipedunculata* inflorescences, should be further studied because of its significant strength against the estrogen-dependent MDA-MD-361 cell line.

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**ЦИТОТОКСИЧНА, АНТИОКСИДАТИВНА И АНТИМИКРОБИЈАЛНА АКТИВНОСТ
AMPELOPSIS BREVIPEDUNCULATA И *PARTHENOCISSUS TRICUSPIDATA* (VITACEAE)**

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Циклохексански и метанолни екстракти листова и цвасти *Ampelopsis brevipedunculata* и *Parthenocissus tricuspidata* показали су значајну цитотоксичну активност на естроген-зависне (MDA-MB-361) и естроген-независне ћелије рака дојке (MDA-MB-453). Метанолни екстракт *P. tricuspidata* испољио је већу цитотоксичност на MDA-MB-453 ћелије (цваст: $IC_{50} = 111.45 \pm 2.56 \mu\text{g/ml}$; листови: $IC_{50} = 56.76 \pm 7.11 \mu\text{g/ml}$) у односу

на MDA-MB-361 ћелије. Циклохексански екстракт листова *A. brevipedunculata* показао је високу цитотоксичност на MDA-MB-453 ($IC_{50} = 78.32 \pm 0.1 \mu\text{g/ml}$), као и на естроген-зависне MDA-MB-361 ћелије ($IC_{50} = 97.40 \pm 2.61 \mu\text{g/ml}$). Највећу способност уклањања DPPH радикала имао је метанолни екстракт цвасти *P. tricuspidata* чија је $IC_{50} = 7.55 \pm 0.07 \mu\text{g/ml}$. Испитивани екстракти поседују слабу антимикуробну активност.