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BIOACTIVE SUBSTANCES IN SHOOTS OF REPRESENTATIVES OF THE GENUS *CELASTRUS* IN THE CONDITIONS OF MOSCOW REGION

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The genus *Celastrus*, or bittersweet, including around 35 species, growing in Asia, Australia, Madagascar and America. Several *Celastrus* species are used mostly as ornamental plants in Russia and abroad. Their ornamental properties include bright leaf coloration in autumn and showy fruits and seeds with orange arils. In addition to ornamental value, bittersweets are known to possess medicinal properties, however, this aspect is currently under-investigated. Some chemical compounds contained in various parts of bittersweets, have shown biological activity. Shoots of representatives of 6 bittersweet taxa, introduced in conditions of Moscow region (*C. orbiculatus*, *C. rugosus*, *C. strigillosus*, *C. flagellaris*, *C. scandens* and *C. orbiculatus* var. punctatus), served as objects in the present study. Samples of frozen leaves and stems kept in a freezer at -10 °C were analyzed by methods of high performance liquid chromatography (HPLC) with ultraviolet (UV) and mass-detection (MS). Spectral data (UV- and MS-spectra) of dominating substances in methanol extracts were obtained and, using literature record on the chemical composition of *Celastrus* plants, the obtained data were analyzed and interpreted. Our investigation demonstrated that taxa of the genus *Celastrus* are producers of chemical constituents with high antioxidant power, such as afzelechin and its dimer and trimer (*C. orbiculatus*, *C. flagellaris*, *C. strigillosus*, *C. orbiculatus* var. puncatus), kaempferol and its glycosides (all studied taxa), quercetin and its glycosides (*C. rugosus*, *C. orbiculatus*, *C. scandens*).

Keywords: Celastrus, shoots, leaves, bioactive substances, Moscow region.

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Introduction

The genus *Celastrus*, or bittersweet, belongs to the family Celastraceae including around 35 species [1–3], growing in Asia, Australia, Madagascar and America. Out of them, 3 species are found in the Russian Far East. They are deciduous, rarely evergreen climbing shrubs, most often – robust vines. Several *Celastrus* species are used mostly as ornamental plants in Russia and abroad. Their ornamental properties include bright leaf coloration in autumn and showy fruits and seeds with orange arils.

In addition to ornamental value, bittersweets are known to possess medicinal properties, however, this aspect is currently under-investigated. Some chemical compounds contained in various parts of bittersweets, have shown biological activity. They include cyclitol (dulcitol from leaves of *C. orbiculatus*) with anti-inflammatory action, catechins – antioxidant, and sesquiterpenoids – antifeeding, antifungal, antiviral and cytotoxic (derivatives of β-agarofuran from roots, fruits and seeds of *C. orbiculatus* as well as over 30 compounds from stems of *C. rugosus*) and also anti-tumor activity (celastrol obtained from *C. orbiculatus* roots) [4–7].

The record on flavonoid composition of bittersweet leaves which contain glycosides of kaempferol and quercetin (*C. orbiculatus* – kaempferol-7-dirhamnoside, kaempferol-3,7-rhamoside, kaempferol-3-glucoside-7-rhamoside,

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quercetin-3,7-dirhamnoside; *C. rugosus*: kaempferol-3-α-L-rhamnoside-7-α-rhamnoside, kaempferol-3-β-glucoside-7-rhamnoside; quercetin-3-β-glucoside-7-α-rhamnoside; *C. scandens*: kaempferol-3-α-rhamnoside-α-rhamnoside, kaempferol-3-β-glucoside-7-α-rhamnoside, quercetin-3-α-glucoside-7-α-rhamnoside) is apparently incomplete [5, 8, 9]. Taxa of this genus contain compounds with potential anti-tumor activity.

Bittersweets are also known to be used directly for medicinal purposes. In Indian and Chinese traditional (folk) medicine fruits of *Celastrus* serve as remedies for rheumatism, uratosis (Gouty arthritis), fever, asthma, stomachache, diarrhea, dysentery, hemorrhoid, snake bites and for better wound healing. Seed oil of *C. paniculatus* is used in India, Asian countries and in the Philippines as a tonic for the brain, to improve memory and promote intellectual development in mentally retarded children, to treat beri-beri disease (wet dropsy), as a wound healing, painkilling and sudorific remedy, for various types of fever, rheumatism and Gouty arthritis; it can also serve as an antidote in case of opioid overdose or as an aphrodisiac agent.

In Russian market, some medications produced in India containing seed oil of *C. paniculatus* (Himkolin gel used for erectile dysfunction) or its extraction (Ayurvedic plant-based preparation Geriforte by Hymalaya Herbals in syrup or tablets) and Geriforte vet (a solution used in veterinary medicine, registration № RK-VP-4-1740-11 of 08.11.11) active as an antioxidant, relaxant and adaptogenic tonic are available [10–12].

C. orbiculatus is indicated for treating paralysis, headache or toothache, snake bites; its roots, stems and leaves possess anti-inflammatory, antirheumatic, purgatory and tonifying properties. The decoction of roots and stems is administered internally use while fresh chopped leaves are for external use only. *C. scandens* was known and used medicinally by native North American herbalists. Folk medicine mostly recommends using roots – fresh or as poultrice, extractions or tinctures as sudorific preparations, diuretics or nauseants. They also work for treating persisting ulcers, skin rashes, chronic liver and skin conditions (including skin cancer), rheumatism, leucorrhea (whites) and dysentery. Its bark is used externally as an ingredient of an oinment for burns, scratches and skin rashes, its extracts are known to have cardiac activity. Care should be exercised when collecting and handling this plant as it is supposedly toxic [13–17].

Materials and methods

Objects. Shoots of representatives of 6 bittersweet taxa – *C. orbiculatus*, *C. rugosus*, *C. strigillosus*, *C. flagellaris, C. scandens* and *C. orbiculatus* var. *punctatus* grown on the grounds of the Tsitsin Main Botanic Garden of Russian Academy of Sciences (MBG RAS) served as objects in the present study. Voucher specimens are kept in the Herbarium of MBG RAS (Table. 1).

Samples (samples of frozen leaves and stems kept in a freezer at -10 °C) were analyzed by methods of high performance liquid chromatography (HPLC) with ultraviolet (UV) and mass-detection (MS). Spectral data (UV- and MS-spectra) of dominating substances in methanol extracts were obtained and, using literature record [4, 18–20] on the chemical composition of *Celastrus* plants, the obtained data were analyzed and interpreted.

Equipment. For UV-HLPC analysis, Shimadzu Prominence I LC-2030C 3D chromatograph was used.

For MS-HLPC analysis, we used Shimadzu LCMS-8045 chromatograph.

Sample preparation. Approximately 0.1 g (precisely weighed quantity) of the plant raw material was placed in an Eppendorf-type microcentrifuge tube and crushed using Qiagen Q-85300 tissue grinder (homogenizer) for 5 min. Then, 1 ml of methanol was added to the homogenized sample and the processing was repeated. Upon that, the roughage was sedimented in an Eppendorf centrifuge at 7000 r.p.m. for 10 min. The resulting supernatant fluid was removed into a vial for subsequent chromatography.

Conditions for chromatography. Table 2 shows the conditions of chromatographic analysis.

An alternative methodology for the studied samples – gas chromatography with mass spectrometric detection – was tested for these objects but the obtained results appeared to be undescriptive.

Table 1. Herbarium voucher specimens of taxa included in the comparative analysis of plant raw material

Voucher specimen number	Taxon	Harvest location
MHA009822	*C. rugosus* Rehder & E.H.Wilson	Arboretum of MBG RAS
MHA0095817	*C. orbiculatus* Thunb.	Arboretum of MBG RAS
MHA0095821	*C. scandens* L.	Arboretum of MBG RAS
MHA0095820	*C. flagellaris* Rupr.	Arboretum of MBG RAS
MHA0095818	*C. strigillosus* Nakai	Arboretum of MBG RAS
MHA0095819	*C. orbiculatus* var. *puncatus* Rehder	Arboretum of MBG RAS

Table 2. Conditions of chromatographic analysis

Mobile phase A	Purified water, 1st class
Mobile phase B	0.1% formic acid solution acetonitrile (MECN) solution
Column	Shimadzu Shim-pack GIST
Column heating oven temperature	45 °C
Flow rate	0.3 ml/min
Detection	UV, 200–800 nm MS, 150–2000 Da
Sample size	1 mcl
Time of chromatography	Around 60
Gradient timetable:	
Time (minutes)	B (%)
0	5
25	30
45	100
50	100
51	5
60	5

Results of chromatographic analysis

Six leaf and six stem samples of the studied bittersweet taxa were analysed by means of the techniques described above. Stem samples were found to contain trace amounts of secondary metabolites, therefore, the concentration in the experimental samples was insufficient to get the UV spectrum.

For leaf samples, chromatograms of methanol extracts' HPLC-MS profiles with negative ionization are given below. For the main peaks, UV-absorption spectra and MS-spectra at positive and negative ionization are provided. The obtained spectral data array coupled with available literature record allowed us to identify the compounds present in the samples with high degree of accuracy.

Table 3 presents chemical constituents found in leaf samples in comparison with the available literature record.

In Table 4, the list of identified phenolic compounds in leaf samples of 6 *Celastrus* taxa is provided.

Table 3. Original and literature data on the qualitative composition of various plant parts in the genus *Celastrus*

Species	Chemical compound	Original experimental data – leaves		Literature record				
		Compounds found in leaves	Compounds not found in leaves	Leaves	Shoots	Roots	Fruits	Seeds
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
C. orbiculatus, (Sample 2)	cyclitols	…	…	+	…	…	…	…
	sesquiterpenoids	…	Celastrine B, Celahin D, Celafolin D-3	…	…	+	+	+
	triterpenoids	…	Paeonenolide G, Oleanolic alcohol, Orthosphenic acid, Celastrol, Olibanumol H, Echinocystic acid, Datura-nolone, b-Amyron, 3-Oxo-11-bhydroxyfriedelane, Ne-opanaxadiol	…	+	…	…	…
	diterpenoids	…	Vitetrifolin E	…	+	+	…	…
	steroids	…	Turkesterone, Chlorogenin, Viticosterone, Seringosterol, Fungisterol, Isofucosterol, Polasterol A, Corbisterin	…	+	…	+	…
	Phenols and their derivates	…	Saucerneol A	…	+	…	…	…
	Phenolcarbonic acids	…	…	…	+	…	…	…
	flavonoids	…	…	+	+	…	…	…

End of table 3

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
C. orbiculatus, (Sample 2)	Flavonoid glycosides	Kaempferol gluco-rhamnoside; Quercetin dirhamnoside	…	+	…	…	…	…
	Catechins (condensed tannins)	Catechin, Afzelechin, Afzelechin dimer, Afzelechin trimer	…	…	+	…	…	…
	lactones	…	…	…	+	…	…	…
	alkaloids	…	…	…	…	…	+	…
	Fatty oil	…	Oleopalmitic acid	…	…	…	…	+
	carotenoids	…	…	No available information on the distribution in the plant body				
C. rugosus (sample 1)	Flavonoid glycosides	Quercetin gluco-rhamnoside; Quercetin dirhamnoside; Quercetin glucoside; Kaempferol dirhamnoside	…	+	+	…	…	…
C. flagellaris (sample 4)	sesquiterpenoids	…	…	…	…	…	…	+
	carotenoids	…	…	…	…	…	…	+
	Flavonoid glycosides	Kaempferol gluco-rhamnoside; Kaempferol dirhamnoside	…	…	…	…	…	…
C. strigillosus (sample 5)	Catechins (condensed tannins)	Afzelechin, Afzelechin dimer, Afzelechin trimer	…	…	…	…	…	…
	Flavonoid glycosides	Kaempferol dirhamnoside	…	…	…	…	…	…
C.orbiculatus var. *puncatus* (sample 6)	Catechins (condensed tannins)	Afzelechin, Afzelechin dimer, Afzelechin trimer	…	…	…	…	…	…
	Flavonoid glycosides	Kaempferol dirhamnoside	…	…	…	…	…	…
C. scandens (sample 3)	celastrol β-amyrin lupeol	…	…	No available information on the distribution in the plant body				
	Flavonoid glycosides	Quercetin digluco-rhamnoside; Kaempferol digluco-rhamnoside; Quercetin gluco-rhamnoside; Kaempferol gluco-rhamnoside	…	+	…	…	…	+

Table 4. Phenolic compounds in leaf sample of *Celastrus* representatives

Retention time, min	Absorption maximum in UV spectrum, nm	Identified molar mass, Da	Identified chemical compound	Leaf sample number*
11.2	353	772	Quercetin digluco-rhamnoside	3
12.1	275	290	Catechin	2
12.7	346	756	Kaempferol digluco-rhamnoside	3
14.5	272	546	Afzelechin dimer	2, 4, 5, 6
14.6	272	274	Afzelechin	2, 5, 6
15.2	272	546	Afzelechin dimer	2, 5, 6
15.3	354	610	Quercetin gluco-rhamnoside	1
16.8	346	594	Kaempferol gluco-rhamnoside	1, 2, 4, 5, 6
17.1	349	594	Quercetin dirhamnoside	1, 2
17.2	354	610	Quercetin gluco-rhamnoside	3
17.8	349	464	Quercetin glucoside	1
18.2	-	818	Afzelechin trimer	2, 5, 6
18.9	343	578	Kaempferol dirhamnoside	1, 4, 5, 6
19.2	347	594	Kaempferol gluco-rhamnoside	3

* 1 – *C. rugosus*, 2 – *C. orbiculatus*, 3 – *C. scandens*, 4 – *C. flagellaris*, 5 – *C. strigillosus*, 6 – *C. orbiculatus* var. puncatus.

Our studies showed that no cyclitols of flavonoids were found in the leaf sample of *C. orbiculatus* as well as in its leaves and stems, no diterpenoids, triterpenoids, phenols or their derivatives, phenolcarbonic acids, flavonoids or lactones were identified. The presence of these chemical constituents was earlier mentioned in relevant literature [1, 2, 8]. Meanwhile, flavonoid glycosides and catechins, earlier recorded in leaves and shoots, were also found in this research. The study revealed the presence of flavonoid glycosides in leaves of *C. rugosus* and *C. scandens*, proving the literature record and specifying the particular substances within these types of compounds. For the first time, in leaves of *C. flagellaris*, *C. strigillosus* and *C. orbiculatus* var. *punctatus* flavonoid glycosides were detected, and catechins – in the latter two. Sesquiterpenoids, revealed in other plant parts in some *Celastrus* species, were not found in the leaves of any species involved in the present study.

Our investigation demonstrated that taxa of the genus *Celastrus* are producers of chemical constituents with high antioxidant power, such as afzelechin and its dimer and trimer (*C. orbiculatus*, *C. flagellaris*, *C. strigillosus*, *C. orbiculatus* var. *puncatus*), kaempferol and its glycosides (all studied taxa), quercetin and its glycosides (*C. rugosus*, *C. orbiculatus*, *C. scandens*) (Table 4). For catechin and afzelechin derivatives, there are indications of their possible antioxidant and anticancer activity [4].

Conclusion

In the course of the present investigation, data on the qualitative chemical composition of leaves in six *Celastrus* taxa: *C. rugosus*, *C. orbiculatus*, *C. scandens*, *C. flagellaris*, *C. strigillosus*, *C. orbiculatus* var. *puncatus* were obtained. The stems of Celastrus did not appear to accumulate any bioactive substances. Flavonoid glycosides were revealed in leaves of all studied *Celastrus* taxa. Catechins were found in leaves of *C. orbiculatus*, *C. strigillosus* и *C. orbiculatus* var. *punctatus.* Notably, in the leaves of *C. strigillosus* and *C. orbiculatus* var. *punctatus*, flavonoid glucosides and catechins are reported for the first time. It was demonstrated that main class of compounds contained in the leaves of the studied taxa were flavonoid glycosides (quercetin and kaempferol derivatives) and condensed tannins (catechin and afzelechin derivatives), the latter known to possess antioxidant and anticancer properties.

Further investigations may be focused on the interpretation of the obtained results in comparison with those of medical and biological studies, to reveal possible correlations.

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Conflict of Interest
The authors of this work declare that they have no conflicts of interest.

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