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Identification of potential sources of thymoquinone and related compounds in Asteraceae, Cupressaceae, Lamiaceae, and Ranunculaceae families

Research Article

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Abstract: In this study, forty-seven plant species belonging to seven families were analysed by GC and GC-MS for the contents of pharmacologically effective quinones: dithymoquinone (DTQ), thymohydroquinone (THQ), and thymoquinone (TQ). The results showed that detectable amounts (≥1 mg kg^{-1}) of at least one of these compounds have been found in three species of both *Monarda* (*M. didyma*, *M. media*, and *M. menthifolia*) and *Thymus* (*T. pulegioides*, *T. serpyllum*, and *T. vulgaris*) genera, two *Satureja* (*S. hortensis* and *S. montana*) species, and in single representatives of *Eupatorium* (*E. cannabinum*), *Juniperus* (*J. communis*), and *Nigella* (*N. sativa*) genera. The maximum contents of THQ and TQ were found in *M. media* aerial parts and *M. didyma* inflorescences (2674 and 3564 mg kg^{-1} of dried weight, respectively) in amounts significantly exceeding their maximum contents in *N. sativa* seeds (THQ = 530 mg kg^{-1} and TQ = 1881 mg kg^{-1}), which are generally considered as the main natural source of both of these compounds. As a conclusion, *M. didyma* (bergamot) and *M. media* (purple bergamot) can be recommended as new prospective natural sources of THQ and TQ for pharmaceutical or food industries.

Keywords: *Dihydrothymoquinone • Monarda • Nigella • Quinones • Thymoquinone*

1. Introduction

Thymoquinone (TQ) is a naturally occurring compound belonging to the benzoquinones (synonym: 2-isopropyl-5-methyl-1,4-benzoquinone), which has previously demonstrated many biological activities in a large number of experiments carried out in molecular, cellular and animal models [1-3] as well as in several clinical studies showing its anti-epileptic effect in children with refractory seizures [4] and confirming that TQ is well tolerated when administered to patients with advanced refractory malignant disease [5]. Although the main

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attention is by its promising anticancer properties, the marked anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, and immunomodulatory effects of TQ were also recorded in experiments usually performed as a part of research focused on assessment of biological activity of *Nigella sativa* L. [6-10], a plant species belonging to the Ranunculaceae family, whose seeds commonly known as black cumin are generally considered as the main natural source of TQ [11].

Besides Ranunculaceae, where trace amounts of TQ were detected also in *Nigella arvensis* L. seeds [12], the presence of this compound has previously been confirmed in several genera of the Lamiaceae family such as *Agastache* [13], *Coridothymus* [14], *Monarda, Mosla* [15], *Origanum* [16-19], *Satureja* [15,20], *Thymbra* [21], and *Thymus* [22-24]. It has also been found in genus *Tetraclinis* [25], and in a glycosidic form in the genera *Cupressus* [26,27] and *Juniperus* [28] of the Cupressaceae family.

In many plant species, TQ is occurring together with its dimeric and reduced forms dithymoquinone (DTQ) and thymohydroquinone (THQ), the second being considered as a compound with prospective antibacterial [29], antifungal [30], anti-inflammatory [31], antioxidative [32], and acetylcholinesterase inhibitory [33] effects. Similarly as TQ, THQ is distributed within a number of genera of the Lamiaceae family such as *Coridothymus* [14], *Origanum* [19], *Monarda*, *Mosla*, *Satureja* [15] and *Thymus* [22,23]. Its presence has also been confirmed in the genera *Tetraclinis* [25] and *Nigella* [29] of the Cupressaceae and Ranunculaceae families, respectively.

Although DTQ produces usually weaker biological activities than THQ and TQ [30-32], several studies indicate its prospective pharmacological properties such as cytotoxicity for human tumour cell lines [34]. This compound has been detected in some species of the Lamiaceae [23] and Ranunculaceae [35] families.

Despite the fact that TQ and its related compounds have been previously detected in a number of plant species, the quantification of their contents has not been previously performed in most published studies. Thus, we decided to determine quantities of DTQ, THQ, and TQ in various plant materials with the aim to identify potential sources of these pharmacologically prospective compounds.

2. Experimental procedure

2.1. Plant material

For the purpose of this study, representatives of seven plant families (Asteraceae, Cupressaceae, Lamiaceae, Oleaceae, Plantaginaceae, Ranunculaceae, and Scrophulariaceae), four of them belonging to the order Lamiales, were analysed. The majority of analysed plant species were grown on the experimental field of the Czech University of Life Sciences Prague (CULS Prague) or at the university campus, several samples were collected in different regions of the Czech Republic from private gardens or wild populations, and the seeds of the *Nigella* genus were obtained from commercial suppliers. The samples of plant material were collected in the period 2007-2011 (mostly in summer and autumn) from one to three independent populations for each species. The total number of analysed samples ranged from one to five for each population according to the accessibility of plants. Additionally, series of samples covering the principal part of one vegetation period (from June to November) were collected for five plant species in the season of 2010 (*Monarda didyma* L., *Satureja hortensis* L., *S. montana* L., *Thymus pulegioides* L., and *T. vulgaris* L.). The voucher specimens authenticated by Dr. Kunt and Dr. Zeleny are deposited at the Department of Chemistry of CULS Prague. Botanical names and voucher specimen numbers (VSNs), plant families, and parts of the plant species containing detectable levels ≥1 mg kg^{-1} (related to dried weight of the sample) of at least one of three quinone compounds (DTQ, THQ and TQ) are summarized in Table 1. The scientific names and VSNs of remaining plants analysed in this study are listed in the second paragraph of the Results and discussion section.

2.2. Reagents & solutions

Butylhydroxytoluene (BHT, 2,6-di-*tert*-butyl-4-methylphenol, ≥99%), carvacrol (≥98%), β-glucosidase from almonds (lyophilized powder, 7.8 units mg^{-1}), octyl-β-D-glucopyranoside (≥99%), thymol (≥99%), and TQ (≥99%) were purchased from Sigma-Aldrich (Prague, Czech Republic). DTQ (purity 98%) was prepared by the dimerization of TQ by the action of daylight using method described in [36]. THQ (purity 96%) was prepared by the reduction of TQ with acetic acid in the presence of zinc powder according to the method described by our team in [32]. As described further, the synthesized reference standards (DTQ and THQ) were analysed by ^{1}H NMR spectroscopy, HPLC-PDA and GC-MS to confirm their identity and purity. The structures of quinones and related compounds are shown in Fig. 1. All reference standards were dissolved in mixture of hexane and isopropyl alcohol (1:1) prior to the GC and GC-MS analyses. If not specified otherwise, all solvents and reagents were obtained from Lach-Ner (Neratovice, Czech Republic).

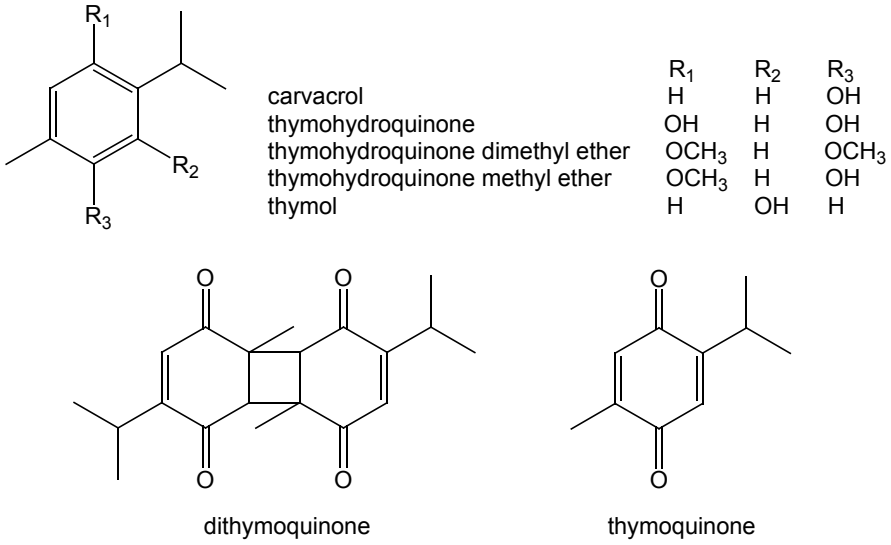

Figure 1. The chemical structures of quinones and related compounds detected in analysed plant samples.

2.3. Extraction of TQ and related compounds

Initially, the air-dried plant material was homogenized using a laboratory mill. Five grams of the powder was then weighed into a paper cartridge and extracted with hexane for 6 hours in the Soxhlet apparatus. Subsequently, the extract was filtered using anhydrous sodium sulphate and re-extracted three times with methanol (30 mL) in a separatory funnel. Finally, the methanol portions were collected in a round bottom flask, methanol was removed in a vacuum rotary evaporator and 10 mL of the internal standard solution (BHT 1 mg mL^{-1}) was added.

2.4. Extraction of glycosides and enzymatic hydrolysis

Additionally, the slightly modified method of extraction and hydrolysis of glycoconjugated quinone forms according to [28] was applied to all samples of the Cupressaceae because glycoconjugated forms of TQ occurring in this family have earlier been reported in literature [26-28]. The extraction cartridge (containing the sample of plant material previously extracted with hexane) was placed into the Soxhlet apparatus and extracted with methanol – water (8:2) for 6 hours. The extract was filtered and purified by shaking twice with hexane; methanol was removed at the vacuum rotary evaporator leaving approximately 40 mL of water solution resting in the flask. Subsequently, 100 mL phosphate buffer (pH 5), 50 mg *β*-glucosidase, and 20 mg of octyl-*β*-D-glucopyranoside (to check the recovery of hydrolysis) were added to the sample, which has been kept at 37°C for 24 hours. The extraction of free aglycones was done three times with ethyl acetate (50, 30, and 30 mL) in a separatory funnel. The extract was dried using anhydrous sodium sulphate, filtered, the solvent was removed in a vacuum rotary evaporator and 10 mL of the internal standard solution (BHT 1 mg mL^{-1}) was added.

2.5. Instrumentation

HPLC-PDA was performed using modified method according to Ghosheh *et al.* [35] on apparatus consisting of SpectraSYSTEM UV6000LP detector (Thermo Finnigan, San Jose, CA, USA), Q-Grad pump (Watrex, Prague, Czech Republic), Mistral column thermostat and Midas autosampler (both Spark Holland, Emmen, The Netherlands). Both compounds (DTQ and THQ) were separated on Biospher PSI 120 (250×4.6 mm, 7 µm) column (Labio, Prague, Czech Republic). Acetonitrile/water phase system was used for gradient elution starting from 20% to 35% acetonitrile in 30min at a flow rate of 1.2 mL min^{-1}. THQ (retention time 8.1 min) and DTQ (retention time 32.7min) were identified according to the comparison of absorption spectra collected at the range of 200-360 nm (local maxima at 290 nm for THQ and 242 nm for DTQ) with those obtained from literature [36,37].

For both substances (DTQ and THQ), ^{1}H (400 MHz), ^{13}C (100 MHz) and 2D (H-COSY, HMQC) NMR spectra were recorded using a Bruker DPX-400 Avance Spectrometer (Bruker BioSpin Corporation, Billerica, MA, USA) in chloroform-d (Sigma-Aldrich, Prague, Czech Republic). Chemical shifts for ^{1}H NMR were referred to trimethylsilane (0 ppm) and for ^{13}C NMR to $CDCl_3$ (77 ppm).

GC analysis were carried out using the gas chromatograph Varian 3300 (Varian, Walnut Creek, CA, USA) equipped with a fused silica capillary column DB-5

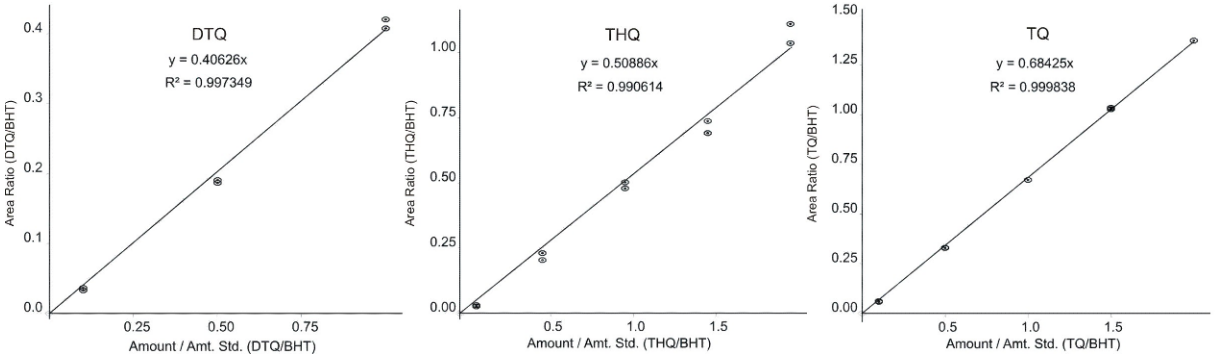

Figure 2. Calibration curves used for quantitative analysis of dithymoquinone (DTQ), thymohydroquinone (THQ), and thymoquinone (TQ).

(30 m × 0.25 mm I. D., film thickness 0.25 µm) and a flame ionization detector (FID). Column temperature programme was 60°C for 2 min, gradient 8°C min^{-1}, upper isotherm 260°C for 8 min; injection port and detector temperature 280°C, split ratio 1:20, carrier gas nitrogen (flow 1 mL min^{-1}).

GC-MS analysis was performed on the gas chromatograph Varian 450-GC (Varian, Santa Clara, CA, USA) equipped with a fused silica capillary column VF-5ms (30 m × 0.25 mm I. D., film thickness 0.25 µm), and connected with the mass spectrometer Varian 240-MS (ion trap, electron ionization energy 70 eV, scan range from 50 to 400 amu). Column temperature programme was 50°C for 2 min, gradient 4°C min^{-1}, upper isotherm 260°C for 15 min; injection port and interface temperature 270°C, split ratio 1:20, carrier gas helium (flow 1 mL min^{-1}).

Identification of compounds was based on Kovats indices (KI) calculated from retention times relative to those of *n*-alkanes (C_8-C_{24}) and on direct comparison with retention times of authentic standards on the DB-5 column (GC-FID) and mass spectra of authentic standards (GC-MS) as well as on those listed in commercial libraries (NIST 05, Wiley) and in the literature [38]. The mass spectra and KI of thymohydroquinone dimethyl ether (THQ-DME, syn.: 2,5-dimethoxy-p-cymene) and thymohydroquinone methyl ether (THQ-ME) were only compared with those in libraries and literature [12,39].

For quantitative analysis of DTQ, THQ and TQ, one microliter of each sample containing 10 mg BHT as an internal standard was injected twice into GC-FID. The contents of compounds were subsequently calculated according to average peak areas. The linearity range of the responses was determined on five concentration levels for THQ and TQ (ranging from 0.1 mg mL^{-1} to 2 mg mL^{-1}) and three concentration levels for DTQ (ranging from 0.1 mg mL^{-1} to 1 mg mL^{-1}) with two injections for each level (keeping the constant concentration of BHT 1 mg mL^{-1}). Calibration graphs for the quantitative evaluation of the compounds were performed by means of a five-point (THQ, TQ) or a three-point (DTQ) regression curves (Fig. 2). Relative standard deviation of sample measurements ranged from 4.4% for THQ to 6.5% for TQ (tested for *M. didyma*, dried aerial parts, n = 3, two GC injections for each sample).

3. Results and discussion

The detectable amounts of TQ, THQ or DTQ have been found in 11 of 47 plant species analysed in this study. Table 1, summarising maximum contents of quinone compounds determined in individual plant taxa, shows that the highest contents of TQ were found in *M. didyma* L. (3564 mg kg^{-1} in inflorescences and 3425 mg kg^{-1} in aerial parts) and *M. media* Willd. (2995 mg kg^{-1} in aerial parts); all exceeding the maximum content of TQ in *N. sativa* L. seeds (1881 mg kg^{-1}). Aerial parts of all the other plant taxa have been observed to contain lower amounts of TQ (ranging from 8 to 1381 mg kg^{-1}) in following manner: *M. menthifolia* Graham, *Satureja montana* L., *M. didyma* L. 'Pink Lace', *Thymus vulgaris* L., *T. serpyllum* L., *T. pulegioides* L., *S. hortensis* L., and *Eupatorium cannabinum* L. The total content of both aglycone and glycosidic forms determined in *Juniperus communis* L. twigs was also significantly lower (21 mg kg^{-1}). Similarly as in the case of TQ, the highest contents of THQ were found in species of *Monarda* genus, however the amount found in *M. media* (2674 mg kg^{-1} in aerial parts) slightly exceeded that of *M. didyma* (2522 mg kg^{-1} in inflorescences and 2409 mg kg^{-1} in aerial parts). Moreover, all *Monarda* species analysed in this study exceeded the maximum amount of THQ in *N. sativa* seeds (530 mg kg^{-1}). Lower contents of THQ (ranging from 47 to 324 mg kg^{-1}) were determined in *S. montana*, *T. vulgaris*, *T. pulegioides*, *S. hortensis*, and *T. serpyllum*. In contrast to both monomeric forms, DTQ was present in several species in very low concentrations only; the

Table 1. Maximum contents of dithymoquinone (DTQ), thymohydroquinone (THQ) and thymoquinone (TQ) in analysed plant species.

Family/Species (voucher specimen number)	Part of plant	NP[1)]	Content (mg kg^{-1} of dried weight)			Note
			DTQ	THQ	TQ	
Asteraceae						
***Eupatorium cannabinum* L. (TB-017)**	Aerial part	1	nd[2)]	nd	8	THQ-DME[3)], THQ-ME[4)] present
Cupressaceae						
***Juniperus communis* L. (TB-002, TB-056)**	Twig	2	nd	nd	6	free form of TQ
			nd	nd	15	glycosidically bound TQ
Lamiaceae						
***Monarda didyma* L., chemotype 1 (TB-005)**	Aerial part	1	128	1811	3029	major component carvacrol
***Monarda didyma* L., chemotype 2 (TB-035)**	Aerial part	1	50	2409	3425	major component thymol
	Inflorescence		58	2522	3564	
	Leaf		nd	466	821	
	Stem		nd	nd	23	
***Monarda didyma* L. 'Pink Lace' (TB-037)**	Aerial part	1	nd	587	670	
***Monarda media* Willd. (TB-072)**	Aerial part	1	34	2674	2995	
***Monarda menthifolia* Graham (TB 073)**	Aerial part	1	nd	835	1381	
***Satureja hortensis* L. (TB-010, TB-044, TB-077)**	Aerial part	3	nd	50	217	
***Satureja montana* L. (TB-011)**	Aerial part	1	62	324	1052	
***Thymus pulegioides* L. (TB-014)**	Aerial part	1	nd	62	223	
***Thymus serpyllum* L. (TB-075)**	Aerial part	1	nd	47	233	
***Thymus vulgaris* L. (TB-015, TB-048)**	Aerial part	2	18	119	300	
Ranunculaceae						
***Nigella sativa* L. (TB-007, TB-067, TB-068)**	Seed	3	38	530	1881	

[1)]number of populations; [2)]not detected (<1 mg kg^{-1}); [3)]thymohydroquinone dimethyl ether; [4)]thymohydroquinone methyl ether

detectable amounts of DTQ ranged from 18 mg kg^{-1} (*T. vulgaris*) to 128 mg kg^{-1} (*M. didyma*).

In contrast to above mentioned species, none of three quinone compounds investigated has been detected in aerial parts of *Aconitum napellus* L. (TB-069), *Aquilegia vulgaris* L. (TB-070), *Chamaecyparis lawsoniana* (A. Murray) Parl. (TB-055), *C. pisifera* (Sieb. & Zucc.) Endl. (TB-081), *Eupatorium rugosum* Kunth. (TB-033), *Helleborus purpurascens* Waldst. et Kit. (TB-071), *Hyssopus officinalis* L. (TB-018), *Juniperus x media* Melle (TB-057), *J. sabina* L. (TB-058), *J. squamata* Buch.-Ham. (TB-059), *Lavandula angustifolia* Mill. (TB-019), *Melissa officinalis* L. (TB-021), *Mentha x piperita* L. (TB-004), *M. x villosa* Huds. (TB-023), *Microbiota decussata* Kom. (TB-060), *Nepeta x faassenii* Bergmans (TB-006), *Nigella damascena* L. (TB-063), *N. sativa* L. (TB-026), *Origanum majorana* L. (TB-003), *O. vulgare* L. (TB-008), *Perilla frutescens* (L.) Britt. (TB-038), *Perovskia atriplicifolia* Benth. (TB-009), *Plantago lanceolata* L. (TB-039), *P. major* L. (TB-040), *P. media* L. (TB-041), *Ranunculus asiaticus* L. (TB-072), *Rosmarinus officinalis* L. (TB-042), *Salvia officinalis* L. (TB-043), *Thuja occidentalis* L. (TB-061), and *Thymus x citriodorus* Schreb. (TB-032), as well as in seeds of *Nigella arvensis* L. (TB-062), *N. damascena*, *N. garidella* Spenn. (TB-065), *N. hispanica* L. (TB-066), and *N. orientalis* L. (TB-067), leaves of *Ligustrum vulgare* L. (TB-053) and *Syringa vulgaris* L. (TB-046), and *Paulownia tomentosa* (Thunb.) Steud. (TB-054) leaves and fruits.

As far as distribution of TQ or its derivatives in plant taxa is considered, their presence has been

Table 2. The main components of hexane/methanol extract from *Monarda didyma* inflorescences (chemotype 2).

Name of compound	Kovats indices		Relative content [%]	Identification[3]
	Measured[1]	Literature[2]		
1-Octen-3-ol	977	978	0.97	MS, KI
p-Cymene	1025	1026	1.26	MS, KI, STD
***cis*-Sabinene hydrate**	1068	1068	1.09	MS, KI
Thymoquinone	1252	1249	13.11	MS, KI, STD
Thymol	1293	1290	62.57	MS, KI, STD
Carvacrol	1301	1298	5.50	MS, KI, STD
Thymohydroquinone	1554	1553	7.84	MS, KI, STD
Dithymoquinone	2359	- [4]	0.14	MS, STD
Total			92.48	

[1]values calculated from retention times on DB-5 column relative to those of C_8–C_{24} alkanes; [2]data taken from [38]; [3]compounds identified according to the mass spectra (MS), Kovats indices (KI) and authentic standards (STD); [4]literature data not available

confirmed in four of seven plant families analysed in this work. The results showed that the presence of at least one of these compounds has been detected in three species of both *Monarda* (*M. didyma*, *M. media*, and *M. menthifolia*) and *Thymus* (*T. pulegioides*, *T. serpyllum*, and *T. vulgaris*) genera, two *Satureja* (*S. hortensis* and *S. montana*) species (all belonging to the Lamiaceae family), and in single representatives of *Eupatorium* (*E. cannabinum*, Asteraceae), *Juniperus* (*J. communis*, Cupressaceae), and *Nigella* (*N. sativa*, Ranunculaceae) genera. Two distinct populations of *M. didyma* included in this study have been identified as two different chemotypes 1 and 2 characterised by major components carvacrol and thymol, respectively. More detailed analysis of chemotype 2 (Table 2 and Fig. 3), which contained slightly higher amounts of TQ and THQ in aerial parts than chemotype 1, showed that the significantly higher contents of these substances were present in inflorescences than in leaves and stems. The only cultivar included into this study (*M. didyma* 'Pink Lace') showed substantially lower amount of TQ and THQ in comparison with both chemotypes.

Although the presence of TQ or related compounds in *M. didyma* as well as in majority of other samples (*N. sativa*, *S. hortensis*, *S. montana*, *T. pulegioides*, *T. serpyllum*, and *T. vulgaris*) analysed in this study has previously been described in literature data [15,20,23,24,35,40], according to the best of our knowledge, this is the first report on presence of TQ in *M. media* and *M. menthifolia*. Moreover, only methylated or glycosidically bound forms have previously been identified in some plant species. For example, we detected low amounts of TQ, THQ-DME, and THQ-ME in *E. cannabinum*. The occurrence of THQ-ME in this species is in agreement with the results of Bellardi and Piccaglia [41] who studied the composition of essential oils from leaves and stems infected by cucumber mosaic virus. However, despite the fact that the presence of THQ-DME has been reported in the genus *Eupatorium* [42], the occurrence of this compound (and TQ) was not previously described in *E. cannabinum*. In another case, we detected TQ and THQ in free form in *T. pulegioides*, whereas Radonic and Mastelic [40] reported only TQ in glycoconjugated form. With the aim to verify if species of the Cupressaceae family can be important sources of quinones, we performed hydrolysis of their glycoconjugated forms as described in [28]. Regrettably, these analyses performed with eight conifer species led to the isolation of low amount of glycoconjugated TQ (15 mg kg^{-1}) from twigs of *J. communis* only.

In only one case, we did not confirm the findings of earlier report describing presence of trace amounts of TQ in the seed essential oil of *N. arvensis* [12]. However, when comparing the analytical data from this study with previously published works on the genus *Nigella*, it is essential to consider differences in the extraction method used, which may significantly influence their chemical composition as previously described for *N. damascena* [43], *N. orientalis* [44], and *N. sativa* [45,46]. Moreover, the significant variations in chemical composition of *N. sativa* seeds have previously been ascribed to the influence of origin of the plant [47] and agronomic techniques used, e. g. sowing date (reported also for *N. damascena*), irrigation regime or fertilizer type [48-50]. The influence of these factors should also be taken into consideration for interpretation of analyses of other species investigated in this study.

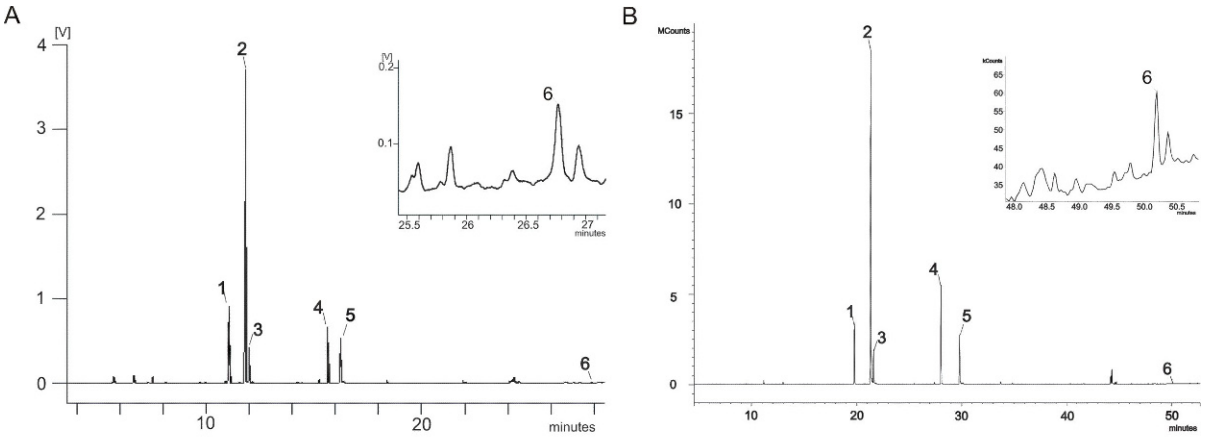

Figure 3. GC-FID (A) and GC-MS (B) chromatograms of *Monarda didyma* (chemotype 2) inflorescences: 1-thymoquinone, 2-thymol, 3-carvacrol, 4-butylhydroxytoluene (internal standard), 5-thymohydroquinone, and 6-dithymoquinone.

4. Conclusions

In this work, new potential sources of TQ and related compounds together with their taxonomic distribution in selected plant families were studied. In total, forty-seven plant species were analysed, eleven of them (namely *E. cannabinum*, *J. communis*, *M. didyma*, *M. media*, *M. menthifolia*, *N. sativa*, *S. hortensis*, *S. montana*, *T. pulegioides*, *T. serpyllum*, and *T. vulgaris*) contained at least one of the quinone substances at the detectable levels. The quantification analysis showed that the highest contents of TQ and THQ were found in inflorescences of *M. didyma* and in aerial parts of *M. media* exceeding approximately 2 and 5 times their respective maximum contents in *N. sativa* seeds, which are generally considered as the main natural source of both of these compounds. Thus, these species commonly known as bergamot or Oswego tea (*M. didyma*) and purple bergamot (*M. media*), which are grown in several varieties as ornamental plants and used in folk medicine for their anthelmintic, carminative, diuretic, expectorant, febrifuge, rubefacient and stimulant properties [51], can be recommended as new prospective natural sources of THQ and TQ, especially for growing under moderate climate conditions. However, further research focused on breeding and selection of the most prospective varieties as well as optimisation of agronomical and processing technologies should be performed for enhancement of potential industrial value of these plants.

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References

[1] H. Gali-Muhtasib, A. Roessner, R. Schneider-Stock, Int. J. Biochem. Cell Biol. 38, 1249 (2006)
[2] S. Banerjee et al., Nutr. Cancer 62, 938 (2010)
[3] C.C. Woo, A.P. Kumar, G. Sethi, K.H.B. Tan, Biochem. Pharmacol. 83, 443 (2012)
[4] J. Akhondian et al., Epilepsy Res. 93, 39 (2011)
[5] A.M. Al-Amri, A.O. Bamosa, Shiraz E-Med. J. 10, 107 (2009)
[6] M.A. Khan, Inflammopharmacology 7, 15 (1999)
[7] B.H. Ali, G. Blunden, Phytother. Res. 17, 299 (2003)
[8] M.L. Salem, Int. Immunopharmacol. 5, 1749 (2005)
[9] M.F. Ramadan, Int. J. Food Sci. Technol. 42, 1208 (2007)
[10] M.A. Randhawa, M.S. Alghamdi, Am. J. Chin. Med. 39, 1075 (2011)
[11] L. Kokoska, Chemistry and Biological Activity of Nigella Genus (LAP Lambert Academic Publishing, Saarbrücken, 2011)
[12] J. Havlik, L. Kokoska, S. Vasickova, I. Valterova, Flavour Frag. J. 21, 713 (2006)
[13] Y.-J. Long, P.-X. Feng, W.-J. Cai, J. Northeast Forestry Univ. 26, 72 (1998)
[14] Z. Fleisher, A. Fleisher, J. Essent. Oil Res. 14, 105 (2002)
[15] J.A. Pedersen, Biochem. Syst. Ecol. 28, 229 (2000)

[16] C. Economakis, H. Skaltsa, C. Demetzos, M. Sokovic, C.A. Thanos, J. Agric. Food Chem. 50, 6276 (2002)
[17] E. Ipek et al., Food Chem. 93, 551 (2005)
[18] B. Lukas, C. Schmiderer, C. Franz, J. Novak, J. Agric. Food Chem. 57, 1362 (2009)
[19] C. Hirobe, Z.S. Qiao, K. Takeya, H. Itokawa, Nat. Med. (Tokyo) 52, 74 (1998)
[20] A. Kubatova, A.J.M. Lagadec, D.J. Miller, S.B. Hawthorne, Flavour Frag. J. 16, 64 (2001)
[21] Z. Fleisher, A. Fleisher, J. Essent. Oil Res. 17, 32 (2005)
[22] F. Mouhajir, J.A. Pedersen, M. Rejdali, G.H.N. Towers, Pharm. Biol. 39, 391 (2001)
[23] M. Jukic, M. Milos, Croat. Chem. Acta 78, 105 (2005)
[24] S. Aziz et al., J. Essent. Oil Bear. Plants 13, 224 (2010)
[25] A.F. Barrero et al., J. Essent. Oil Res. 17, 166 (2005)
[26] M. Milos, A. Radonic, Acta Pharm. (Zagreb) 46, 309 (1996)
[27] M. Milos, J. Mastelic, A. Radonic, Croat. Chem. Acta 71, 139 (1998)
[28] J. Mastelic, M. Milos, D. Kustrak, A. Radonic, Croat. Chem. Acta 73, 585 (2000)
[29] M.A. Toama, T.S. El-Alfy, H.M. El-Fatatry, Antimicrob. Agents Chemother. 6, 225 (1974)
[30] K. Halamova et al., J. Food Protect. 73, 2291 (2010)
[31] P. Marsik et al., Planta Med. 71, 739 (2005)
[32] H. Tesarova et al., Nat. Prod. Commun. 6, 213 (2011)
[33] M. Jukic, O. Politeo, M. Maksimovic, M. Milos, M. Milos, Phytother. Res. 21, 259 (2007)
[34] D.R. Worthen, O.A. Ghosheh, P.A. Crooks, Anticancer Res. 18, 1527 (1998)
[35] O.A. Ghosheh, A.A. Houdi, P.A. Crooks, J. Pharm. Biomed. Anal. 19, 757 (1999)
[36] L.I. Smith, R.W.H. Tess, J. Am. Chem. Soc. 66, 1323 (1944)
[37] M. El-Dakhakhny, Planta Med. 11, 465 (1963)
[38] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, 4th Edition (Allured Publishing Corporation, IL, 2007)
[39] H.L. De Pooter, L.F. De Buyck, N.M. Schamp, F.M. Harraz, I.M. El-Shami, Flavour Frag. J. 6, 157 (1991)
[40] A. Radonic, J. Mastelic, Croat. Chem. Acta 81, 599 (2008)
[41] M.G. Bellardi, R. Piccaglia, Agricol. Mediterr. 135, 173 (2005)
[42] A. Gauvin-Bialecki, C. Marodon, Biochem. Syst. Ecol. 36, 853 (2009)
[43] A. Wajs, R. Bonikowski, D. Kalemba, Nat. Prod. Commun. 4, 1577 (2009)
[44] A. Wajs, D. Kalemba, J. Essent. Oil Res. 22, 232 (2010)
[45] A. Stoyanova, E. Georgiev, A. Wajs, D. Kalemba, J. Essent. Oil Bear. Plants. 6, 207 (2003)
[46] L. Kokoska et al., J. Food Prot. 71, 2475 (2008)
[47] B. Nickavar, F. Mojab, K. Javidnia, M.A.R. Amoli, Z. Naturforsch. 58c, 629 (2003)
[48] L.F. D'Antuono, A. Moretti, A.F.S. Lovato, Ind. Crop. Prod. 15, 59 (2002)
[49] K.A. El-Sayed et al., Saudi Pharm. J. 8, 175 (2000)
[50] F.S. Mozaffari, M. Ghorbanli, A. Babai, M.F. Sepehr, J. Essent. Oil Res. 12, 36 (2000)
[51] S. Foster, J.A. Duke, A Field Guide to Medicinal Plants and Herbs, 2nd edition (Houghton Mifflin Company, Boston, 2000)