

# Antioxidant activity of some coumarins

## Antioxidačná aktivita niektorých kumarínov

Original research article

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**Abstract** Nineteen derivatives of coumarin were tested on the scavenging of 2,2-diphenyl-1-picrylhydrazyl, hydroxyl and superoxide anion radicals. It was found that antioxidant activity exhibits only such coumarins that contain hydroxyl groups. The derivatives without hydroxyl group showed very low antioxidant effectiveness or they were ineffective. On the other hand, the greatest antioxidant effectiveness was exhibited by coumarin derivatives that contained hydroxyl groups in 6 or 8 position, whereas the effectiveness of derivatives with one hydroxyl group in 4, 5 or 7 position was very low. Based on scavenging of the above-mentioned radicals, it was found that the most effective scavengers were 7,8-dihydroxy-4-methylcoumarin (i.e. compound that contains two hydroxyl groups in 7 and 8 positions), (7,8-dihydroxy-2-oxo-2H-chromen-4-yl)acetic acid (this compound contains in addition to two hydroxyl groups in 7 and 8 positions also one hydroxyl group in the acidic residue), esculetin (6,7-dihydroxycoumarin) and 6,7-dihydroxy-4-methylcoumarin.

**Slovak abstract** Otestovali sme devätnásť derivátov kumarínu, na vychytávanie DPPH, hydroxylových, a superoxidových aniónových radikálov. Ich antioxidačná účinnosť bola závislá od počtu a lokalizácie hydroxylových skupín. Deriváty, ktoré nemali žiadnu hydroxylovú skupinu boli veľmi slabo účinné, alebo vôbec neúčinné. Najúčinnnejšie vychytávače radikálov boli tie deriváty kumarínu, ktoré obsahovali hydroxylové skupiny v polohe 6 alebo 8. Na druhej strane deriváty, ktoré mali iba jednu hydroxylovú skupinu v polohe 4, 5 alebo 7 boli veľmi slabo účinné. Najúčinnnejšie vychytávače vyššie spomínaných radikálov boli 7,8-dihydroxy-4-metylkumarín (zlúčenina, ktorá obsahuje 2 hydroxylové skupiny na benzénovom jadre v polohe 7 a 8), 7,8-dihydroxykumarín-4-yl-octová kyselina (obsahuje okrem 2 hydroxylových skupín na benzénovom jadre má aj OH skupinu v kyselinovom zvyšku, eskuletín (6,7-dihydroxykumarín) a 6,7-dihydroxy-4-metylkumarín.

**Keywords** coumarins – DPPH – EPR – hydroxyl – superoxide anion radicals – scavenger activity

**Kľúčové slová:** kumaríny – DPPH – EPR – hydroxyl – superoxid anión radikály – skavengerová aktivita

## INTRODUCTION

Coumarins are compounds with extensive biologic activities. They can be found to occur naturally in a lot of plants (green beans, lavender, licorice/liquorice, apricots, strawberries, cherries, aloe), also in bacteria and fungi. Coumarins were long ago used as spices and aromatic additives in traditional medicine. Some natural polyphenolic coumarins show anti-inflammatory, antimicrobial, antiviral, anticancer, anticoagulant (as rodenticides) and antioxidant activity (Bentley 1972; Borges *et al.*, 2005; Kostova *et al.*, 2011; Leung *et al.*, 2005; Pedersen *et al.*, 2007; Valchev *et al.*, 2008). Coumarins act also as pesticides in the plants that produce them (Hoult & Payá, 1996; Murray *et al.*, 1982). It was found that esculetin retards senescence and decreases lipid peroxidation (Martin-Arago *et al.*, 1998) protects human

hepatoma HepG2 cells from hydrogen peroxide-induced oxidative damage (Subramaniam & Ellis 2011). Chemical terms of coumarin are 1- benzopyran-2-one or 2H-chromen-2-one. Some coumarins that can be found to occur naturally are umbelliferone (7-hydroxycoumarin is used in sunscreen), esculetin (6,7-dihydroxycoumarin), warfarin and imperatorine (acting as anticogulants), herniarine (acts as antiallergic or fungicide), psoralen (used for psoriasis treatment). The goal of this work is to determine the antioxidant activity (by scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and superoxide anion radicals) of some natural and synthetic coumarins and to investigate the relation between their antioxidant efficiency and structure.

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## MATERIALS AND METHODS

### Reagents and equipments

DPPH, 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), esculetin, 2-mercaptoethanol,  $\beta$ -nicotinamide adenine dinucleotide sodium salt (NADH) were delivered by Sigma-Aldrich Ltd. Methanol p.a., EDTA,  $\text{MnCl}_2$ ,  $\text{FeSO}_4$  and 30%  $\text{H}_2\text{O}_2$  were from the Centralchem firm (Slovakia). Further coumarin derivatives were prepared according to the work of Furdik *et al.*, 1957. Absorption spectra were recorded using spectrophotometer Genesis 6 (Thermo–Scientific, USA). Electron paramagnetic resonance (EPR) spectra were recorded by EPR spectrometer ERS 230 (ZWG Berlin, Germany), which works in X region (approximately 9.3 GHz), with amplitude modulation 0.1 mT, at microwave power 5 mW.

### DPPH assay

Scavenging of DPPH radicals by studied coumarins was monitored by the method, which was described in our previous work Šeršeň *et al.*, 2005. Various amounts of tested compound were added into methanol solution of DPPH and the resulting DPPH concentration was kept constant ( $c = 10^{-4}$  mol  $\text{dm}^{-3}$ ). Twenty minutes after the addition of the tested substance, absorbance was measured at 517 nm.

### Hydroxyl radical assay

Scavenging of hydroxyl radicals was monitored by the method described in our previous works (Valentová *et al.*, 2005, Šeršeň *et al.*, 2005). Hydroxyl radicals were generated by Fenton reaction using  $\text{H}_2\text{O}_2$  and  $\text{FeSO}_4$ . Because  $\text{HO}^\bullet$  radicals are very unstable, it is impossible to determine them directly by continual wave EPR spectrometer. Therefore,  $\text{HO}^\bullet$  radicals captured by the spin trap DMPO and EPR spectra of spin adduct  $\text{DMPO} + \text{OH}^\bullet$  were recorded. Reaction solution was phosphate buffer (0.19 mmol  $\text{dm}^{-3}$   $\text{Na}_2\text{HPO}_4$ , pH = 7.4.), which contained  $5 \cdot 10^{-4}$  mol  $\text{dm}^{-3}$   $\text{FeSO}_4$ , 0.025 mol  $\text{dm}^{-3}$  DMPO, 0.05 mol  $\text{dm}^{-3}$   $\text{H}_2\text{O}_2$  and 0–1 mg  $\text{cm}^{-3}$  of the tested substance. EPR spectra of these solutions prepared in this way were recorded 20 minutes after the addition of  $\text{H}_2\text{O}_2$ .

### Superoxide anion radical assay

Scavenging of superoxide anion radicals ( $\text{O}_2^{\bullet-}$ ) was monitored according to the work of Valentová *et al.*, 2005.  $\text{O}_2^{\bullet-}$  radical was generated from  $\text{O}_2$  in the presence of EDTA,  $\text{MnCl}_2$ , NADH and 2-mercaptoethanol. The reaction mixture contained 0.8  $\text{cm}^3$  of phosphate buffer (19 mmol  $\text{dm}^{-3}$   $\text{Na}_2\text{HPO}_4$ , pH = 7.4), and the tested sample (0.1  $\text{cm}^3$  with concentration 0.0001–1 mmol  $\text{dm}^{-3}$ ) was incubated for 20 minutes. The reaction was started with the addition of 2-mercaptoethanol (0.1  $\text{cm}^3$ , 10 mmol  $\text{dm}^{-3}$ ). Twenty minutes after the addition of 2-mercaptoethanol a decrease of absorbance at 340 nm was recorded.

From the concentration dependencies were calculated  $\text{SC}_{50}$  values, i.e. concentrations of the studied compound that decrease the absorbance or the intensity of the EPR signal to the value of 50% with respect to the control. All measurements were carried out at laboratory temperature.

## RESULTS

Scavenging of DPPH radicals by studied compounds is illustrated in Table 1. From this table, it is evident that coumarin derivatives without hydroxyl groups in benzene ring have very low activity (compounds No. 6, 10, 12–15) or they are ineffective (compounds No. 10, 16–19). On the other hand, coumarins that contain one OH group exhibit very low antioxidant activity (compounds No. 5, 7, 11) or they are ineffective (compound No. 8). Coumarins with 2 hydroxyl groups in positions 7,8 (compounds No. 1 and 2) or 6,7 (compounds No. 3 and 4) exhibit very good antioxidant efficiency, whereas compound (No. 9) with 2 hydroxyl groups in positions 5,7 has very low activity.

Scavenging of  $\text{HO}^\bullet$  radicals was documented by EPR spectroscopy. Fig. 1 shows typical spectra of DMPO adduct

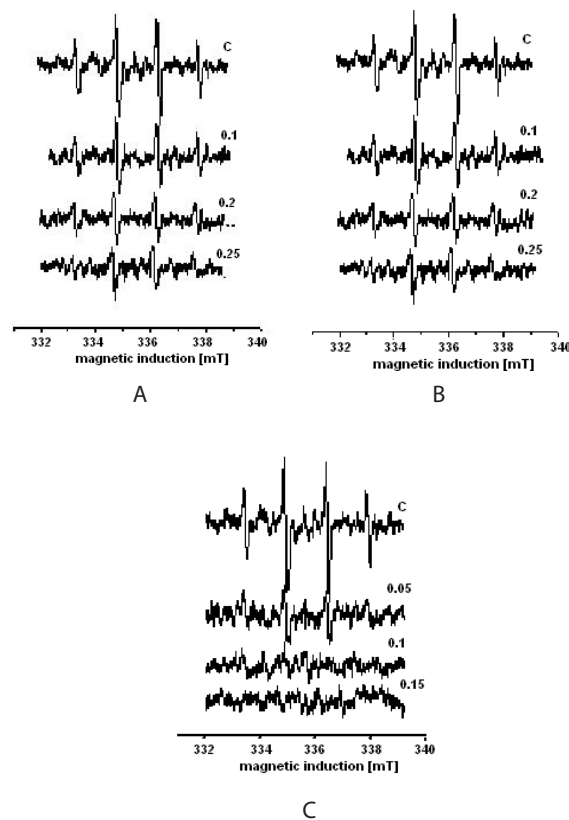


Figure 1. EPR Spectra of spin adduct of DMPO with hydroxyl radicals at the presence of different amount (in mg) 7,8-dihydroxy-4-methylcoumarin (A), 6,7-dihydroxycoumarin (B) and (7,8-dihydroxy-2-oxo-2H-chromen-4-yl)acetic acid (C).

Table 1.  $SC_{50}$  values of coumarins determined by scavenging DPPH radicals

No.	Compound	$SC_{50}$ $\mu\text{mol dm}^{-3}$	$r^2$	Name
1		6.31	0.999	(7,8-dihydroxy-2-oxo-2H-chromen-4-yl)acetic acid
2		8.43	0.973	7,8-dihydroxy-4-methylcoumarin
3		8.64	0.987	6,7-dihydroxycoumarin (esculetin)
4		10.36	0.987	6,7-dihydroxy-4-methylcoumarin (4-methylesculetin)
5		4125	0.616	7-hydroxycoumarin (umbelliferone)
6		5448	0.996	2H-chromen-2-one (coumarin)
7		796	0.997	4-hydroxycoumarin
8		ineffective		7-hydroxy-4-methylcoumarin
9		870	0.773	5,7-dihydroxy-2-oxo-2H-chromen-4-carboxylic acid
10		ineffective		2-oxo-2H-chromen-3-carboxylic acid
11		247/0.917		5-hydroxy-7-methoxy-2-oxo-2H-chromen-4-carboxylic acid
12		4021	0.621	5,7-dimethoxy-2-oxo-2H-chromen-4-carboxylic acid
13		1106	0.958	6,8-dibromo-3-((2,6-dimethylphenyl)thio)-2H-chromen-2-one
14		1349	0.912	2-(3-oxo-3H-benzo[f]chromen-1-yl) acetic acid
15		2073	0.922	2-((2-oxo-2H-chromen-3-yl)oxy)benzoic acid
16		ineffective		N-(2-oxo-2H-chromen-3-yl)benzamide
17		ineffective		2-(2-(2-oxo-2H-chromen-3-yl)acetyl)benzamide
18		ineffective		6-bromo-3-((4-nitrophenyl)thio)-2H-chromen-2-one
19		ineffective		7-methyl-4-((2-oxo-2H-chromen-3-yl)thio)-2H-chromen-2-one

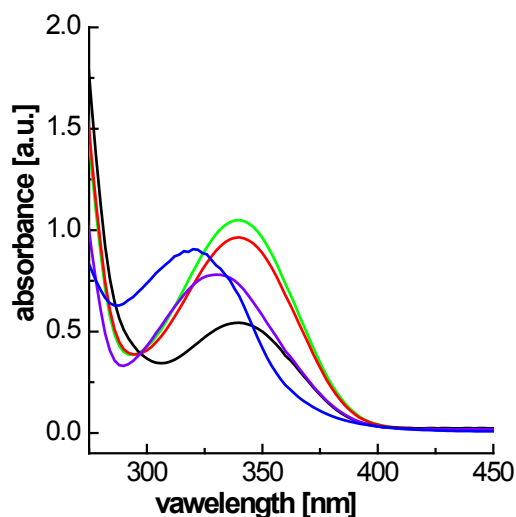


Figure 2. Absorption spectra of NADH (green line), in the presence of  $O_2^{\cdot-}$  radicals without coumarins (black line), and in the presence of  $2.8 \cdot 10^{-5} \text{ mol dm}^{-3}$  esculetin (red line), 7,8-dihydroxy-4-methylcoumarin (blue line) and (7,8-dihydroxy-2-oxo-2H-chromen-4-yl)acetic acid (violet line).

with  $HO^{\cdot}$  radical. Effect of the antioxidant is documented as decrease of intensity of EPR signal.  $SC_{50}$  values were calculated as concentration causing the 50% decrease of intensity of the second line in the EPR spectrum.

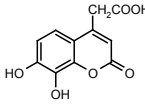
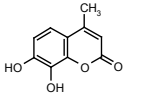
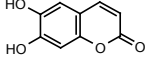
Scavenging of  $O_2^{\cdot-}$  radicals was documented by absorption spectra of NADH solutions. The intensity decrease of absorption band at 340 nm is caused by the oxidation of NADH by the superoxide anion radical. This decrease is lower in the presence of antioxidants, which is documented in Fig. 2.  $SC_{50}$  values were calculated as concentration causing the decrease absorbance at 340 nm to 50% against to control sample.

Antioxidant efficiency of the most active coumarins expressed as the ability of scavenging DPPH, hydroxyl and  $O_2^{\cdot-}$  radicals is presented in Table 2.

## DISCUSSION

We tested 19 coumarin derivatives on antioxidant activity. It was found that their antioxidant efficiency was determined by the number and the position of hydroxyl group in their benzene ring. From Table 1, it is evident that the best scavengers of DPPH radicals are coumarins that have hydroxyl group in the position 6 or 8 in their structure. Their efficiency decreased in following order: 7,8-dihydroxycoumarin-4-yl-acetic acid > 7,8-dihydroxy-4-methylcoumarin > 6,7-dihydroxycoumarin > 6,7-dihydroxy-4-methylcoumarin. The other coumarins that had hydroxyl group in the position 4 (compound No. 7), 5 (compound No. 11) or 7 (compound

Table 2. Scavenging of DPPH,  $HO^{\cdot}$  and  $O_2^{\cdot-}$  radicals expressed through  $SC_{50}$  values of coumarins ( $\mu\text{mol} \cdot \text{dm}^{-3}$ ).

No.	Compound	DPPH/ $r^2$	$HO^{\cdot}/r^2$	$O_2^{\cdot-}/r^2$
1		6.31/0.9989	7.43/0.939	19.21/0.830
2		8.43/0.93	9.18/0.913	29.56/0.969
3		8.64/0.9867	12.1/0.923	57.04/0.911

$r^2$  - the average square deviation

No. 8) showed very low antioxidant efficiency. On the other hand, the efficiency of derivatives without hydroxyl groups was also very low or such derivatives were ineffective.

Some of the studied coumarins (No. 2 - 8) have been previously tested for antioxidant activity; the remaining 12 substances (No. 1, 9 - 19) are tested for the first time. Comparing the DPPH scavenging activity of some coumarins in this study (7,8-dihydroxy-4-methylcoumarin, esculetin, 4-methylesculetin, umbrelliferone, coumarin, 4-hydroxycoumarin and hymecromone) with results of other authors (Hoult & Payá 1996, Panteleon *et al.*, 2003, and Rehakova *et al.*, 2008), it was found that our results are in good agreement with those of the authors mentioned above. Somewhere there were any differences among  $SC_{50}$  values obtained by us and those obtained by those authors, these could be caused by different measurement methods. However, the tendency between their structure and antioxidant activity are identical. Morabito *et al.*, 2010 found that 4-methyl coumarins that had the OH groups, in positions 7 and 8, substituted acetoxy groups showed lower antioxidant activity than those with OH groups. This finding is in accordance with our results since coumarins without hydroxyl groups were ineffective or they exhibited very low antioxidant activity. Our results are also in agreement with the works of Pedersen *et al.*, 2007, Barzegar *et al.*, 2011 and Razo-Hernandez *et al.*, 2014 who found that the most active scavengers of DPPH and galvinoxyl radicals were 6,7- and 7,8-dihydroxy-4-methyl coumarins. Also, Kaneko *et al.*, 2001 found that esculetin bearing a 6,7-dihydroxyl moiety showed a protective effect on linoleic acid hydroperoxide-induced toxicity, whereas 4- and 7- hydroxycoumarins were ineffective. From the structure-activity studies by Payá *et al.*, 1993 and Morabito *et al.*, 2010, the importance of an ortho-dihydroxyl function, especially at the position 7 and 8 can be deduced. Based on the results of tests concerning DPPH scavenging, the most efficient derivatives of coumarin were chosen and these were tested on scavenging of hydroxyl and superoxide

anion radicals. Tested compounds were 7,8-dihydroxy-4-methylcoumarin (a compound that contains 2 hydroxyl groups on the benzene ring in the 7 and 8 positions), 7,8-dihydroxycoumarin-4-yl-acetic acid (compound that contains besides two hydroxyl groups on the benzene ring also an OH group in the acid rest) and 6,7-dihydroxycoumarin and their antioxidant efficiency is presented in Table 2. From this table, it is evident that these coumarins are also very good scavengers also hydroxyl and  $O_2^{\cdot-}$  radicals. Our findings are in accordance with those of Panteleon *et al.*, 2003 and Lin *et al.*, 2008, who found scavenging of hydroxyl radicals by esculetin and 4-methyl esculetin. It was confirmed that coumarins with OH groups in positions 7 and 8 were very good scavengers of  $O_2^{\cdot-}$  radicals (see Table 2). A similar effect was also observed by Hoult & Payá 1996, and Morabito *et al.*, 2010, that is, the most effective coumarins were dihydroxy coumarins, in particular those that contained OH groups in positions 7 and 8.

## CONCLUSION

It was found that from the aspect of antioxidant activity of coumarins, the most important factor is the number and the position of hydroxyl groups on the coumarin skeleton. The most effective coumarins contained two hydroxyl groups on the benzene ring, especially in positions 7 and 8. Coumarins with one or without hydroxyl groups showed very low antioxidant activity or were inactive. Also, it was found that the most effective scavengers of DPPH radicals were also very good scavengers of hydroxyl and superoxide anion radicals.

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