Arabian Journal of Chemistry (2021) **14**, 103068

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Antioxidant activity of some organosulfur compounds *in vitro*

Victoria Osipova [a,*], Maria Polovinkina [b], Yulia Gracheva [c], Dmitry Shpakovsky [c], Anastasia Osipova [b], Nadezhda Berberova [b]

[a] *Southern Scientific Center, Russian Academy of Sciences, Str. Chehova 41, Rostov-on-Don 344006, Russia*
[b] *Astrakhan State Technical University, Str. Tatishcheva 16, Astrakhan 414025, Russia*
[c] *Department of Chemistry, Lomonosov Moscow State University, Leninskie gory 1-3, Moscow 119991, Russia*

Received 9 December 2020; accepted 7 February 2021
Available online 19 February 2021

KEYWORDS

Organosulfur compounds;
Antioxidants;
Radical scavenging activity;
Metal chelating;
Cis-9-octadecenoic (oleic) acid;
Liver of Russian sturgeon

Abstract The medicinal properties of many natural plant products are related with the antioxidant action of their constituents- oligosulfides and polyphenols. To increase the effectiveness of the antioxidant effect in lipid peroxidation and for reducing of undesirable toxic effects the combination in one molecule of several functional groups that act by various mechanisms seems one profitable. The goal of present study was to determine the antioxidant properties of synthetic sterically hindered 2,6-di-*tert*-butyl-4-mercaptophenol (**1**), bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)disulfide (**2**) in comparison with disulfides containing in plant products: diphenyl disulfide (**3**), dibenzyl disulfide (**4**), dibutyl disulfide (**5**), di-*tert*-butyl disulfide (**6**), diallyl disulfide (**7**), methyl propyl trisulfide (**8**). Antioxidant properties were investigated by various assays including DPPH radical scavenging, Cu^{2+} ion reduction (CUPRAC-test), model reactions of peroxidation of *cis*-9-octadecenoic (oleic) acid and lipids from the liver of Russian sturgeon *in vitro* as well as metal chelating activity. It was found that bis(3,5-di-*tert*-butyl-4-hydroxyphenyl) disulfide demonstrated both high DPPH radical scavenging activity and $TEAC_{CUPRAC}$ value and significantly decreased the level of primary products of lipid peroxidation (LPO) - lipid hydroperoxides (LOOH) as well as secondary products - carbonyl compounds which form colored complexes with thiobarbituric acid (TBARS). All sulfur compounds demonstrated the inhibitory activity in oleic acid peroxidation except dibutyl disulfide. The maximum decrease of LOOH and TBARS in lipid peroxidation was detected for bis(3,5-di-*tert*-butyl-4-hydroxyphenyl) disulfide**.** In the case of long-term LPO of liver lipids the antioxidant activity of all sulfur-containing compounds except diphenyl disulfide that exhibit pro-oxidant activity was ascertained. The efficiency of the antioxidant action of most compounds was decreased with time. Compound **2** was the only exception for which the increase in the efficiency of antioxidant

* Corresponding author.
E-mail address: osipova_vp@mail.ru (V. Osipova).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2021.103068

action over time was discovered due to the presence of both 2,6-di-*tert*-butylphenol- and -S-S- fragments providing the prolonged antioxidant action. The high antioxidant efficiency let one to consider compound **2** for practical use as an effective inhibitor of lipid peroxidation in biological substrates and cytoprotector.

1. Introduction

Reactive oxygen species (ROS) formation is an important process in functioning of living organism, while the excessive production of ROS and decrease of antioxidant content leads to the development of oxidative stress, causes oxidative damages of molecular cellular structures (carbohydrates, nucleic acids, lipids, proteins) and changes their functions that ultimately leads to cell death (Munné-Bosch and Pintó-Marijuan, 2017). It is believed that the development of many diseases, including cancer (Waris and Ahsan, 2006), neurodegenerative disorders (Klein and Ackerman, 2003), liver damage (Li et al., 2015), aging (Lee et al., 2004), atherosclerosis (Finkel, 2005), hypertension, ischemia/perfusion, diabetes, chronic obstructive pulmonary disease and asthma (Pham-Huy et al., 2008) are closely associated with oxidative stress. To prevent the effects of oxidative stress, antioxidants of synthetic and natural origin are widely used. Antioxidants in low concentrations can neutralize ROS in various pathways (Green and Shuaib, 2006; Ziakas et al., 2006; Stavrovskaya and Kristal, 2005). Particular interest is directed to natural products that have antioxidant properties (Zhu et al., 2004; Anyasor et al., 2017; Diniyah et al., 2020). Proteins from cereals were found to reduce oxidative stress in cell cultures, animal models, and food systems. Wheat, oat and rice germ proteins showed antiradical activity in the DPPH test (Esfandi et al., 2019; Tsopmo, 2015). The substituted 2,6-dialkylphenols are applied as Vitamin E mimetics (Denisov, 1995). The neuroprotective properties of some phenols including the naturally occurring compounds thymol (5-methyl-2-isopropyl-phenol), carvacrol (2-methyl-5-isopropyl-phenol), eugenol (2-methoxy-4-prop-2-enyl-phenol) as structural analogues of widely used intravenous anesthetic propofol (2,6-bis(isopropyl)-phenol) were found to correlated with their antioxidant capacity. An oxidative injury model using H_2O_2 was applied to evaluate the possible neuroprotective effect of compounds (Delgado-Marín et al., 2017).

Unfortunately, many pharmacological drugs with antioxidant properties that are used in the treatment of pathologies associated with oxidative stress are either insufficiently effective or exhibit side effects. For instance, melatonin treatment may be problematic due to its various physiological roles (Gilgun-Sherki et al., 2002). The negative side effects of various phenolic antioxidants attributed to ability to bind and precipitate proteins (Yehye et al., 2015). Synthetic antioxidants BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) are efficient radical scavengers, but their toxicity and ability to damage different tissues and to promote cancer makes doubtful their usefulness for medical application (Denisov and Afanas'ev, 2005; Final Report on the Safety Assessment of BHT, 2002; Kahl and Kappus, 1993).

Plants have historically been used in treatment of various diseases, including cancer (Adewole, 2020). Alkaloids, terpenoids, polysaccharides and flavonoids from natural products can be used for stabilization of immune system without the side effects of chemotherapeutic drugs. (Parsaeimehr et al., 2018). Biologically active components of natural plant products are constantly tested during experimental and clinical trials since they can alleviate and prevent various pathological conditions (Stefanucci et al., 2020).

Plant foods containing different organosulfur compounds intensively tested as potential anti-inflammatory, anticancer, cardioprotective agents and antioxidants (Cerella et al., 2011; Kimura, 2015). For example, it has been shown that the essential oils of the bark of the stems Drypetes gossweileri (Euphorbiaceae), the roots of Pentadiplandra brazzeana (Capparidaceae), the red bulbs Allium cepa, Alium sativum (Liliaceae) and garlic are rich in phenolic and organosulfur compounds alliin, allicin, ajoene ((E, Z)-4,5,9-trithiadeca-1,6, 11-triene-9 oxide), S-allylcysteine, diallyldisulfide, diallyltrisulfide, 1,2-vinyldithiin that are responsible for the manifestation of high antioxidant, anti-inflammatory, anticancer, antidiabetic, anti-allergic activities, cardio- and hepatoprotective properties (Ndoye Foe et al., 2016; Martins et al., 2016; Colín-González and Santamaría, 2017; Ryu and Kang, 2017; Petrovic et al., 2018; Nicastro et al., 2015). Essential oil from Allium ascalonicum L. containing diallyl disulfide was suggested as natural antibacterial agent (Mahmoudi et al., 2013). The volatile constituents from the Adenocalymma alliaceum contain diallyl disulfide (leaves: 34.0%; flowers: 49.7%), and diallyl trisulfide (leaves: 58.2%; flowers: 32.7%). Dibenzyl disulfide (18.0%) was detected in inflorescences of Petiveria alliacea (Zoghbi et al., 2002).

The authors (Pradeep and Srinivasan, 2017) found that onion (Allium cepa) and fenugreek seeds (Trigonella foenum-graecum) rich in organosulfur compounds have a beneficial effect on hyperglycemia and associated metabolic disorders, which is explained by the synergism of the antiradical, antioxidant action of flavonoids, mainly quercetin, and organosulfur compounds (alk(en)ylcysteine sulfoxides). However, the use of antioxidant cocktails in medical practice is not always convenient and the total concentration of all compounds is so high that it can contribute to the manifestation of undesirable effects. The benefit for human health depends not only on the nature and dose of consumed natural substance, but also on their bioavailability. Among the important factors is the ability of phytocompounds to dissolve or disperse in gastrointestinal fluids. In particular, for lipophilic compounds it is desirable to emulsify them in digestive fluids (Torres-Palazzolo et al., 2021).

In this regard, it is important to create new hybrid molecules - synthetic analogues of natural compounds, combining various biomimetic redox-active functional groups in one structure, which can help one to increase the effectiveness of antioxidant action and reduce side effects. It is also important

not only to explore compounds with targeted pharmacological activity but to identify in this series of compounds that ones which specifically act on certain links of the free radical process in general and on lipid peroxidation in particular.

We have previously synthesized complexes Ph_3SnSR and Ph_3PAuSR based on 2,6-di-*tert*-butyl-4-mercaptophenol (**1**, RSH). Compounds were found to be radical scavengers, Cu^{2+} reducing agents and effective inhibitors of induced mitochondrial lipid peroxidation. The introduction of hindered phenol fragment decreased the cytotoxicity of complexes (Milaeva et al., 2020). Moreover, the antiradical activity of organosulfur compounds 2,6-di-*tert*-butyl-4-mercaptophenol **(1),** bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)disulfide **(2)** and diphenyl disulfide **(3)** toward superoxide radical anion ($O_2^{-\cdot}$) as one of the reactive oxygen species was established (Osipova et al., 2019).

Since the therapeutic effects are related in some extent with the antioxidant action of organosulfur- and phenol derivatives, there is need to proceed out studies using additional model systems in order to identify more effective compounds and to establish the mechanism of their inhibitory action.

In the present work the antioxidant properties of synthetic organosulfur compounds **1** and **2** bearing 2,6-di-*tert*-butylphenol fragments are compared with disulfides **3**–**7** and trisulfide **8** some of them are contained in plant products (Fig. 1). The hindered phenol fragments in **1** and **2** are responsible for the increase of antioxidant activity due to antiradical properties of the *HO*- and/or *HS*-group, chelating and antiperoxide activities of the S atom.

2. Material and methods

2.1. General procedures and syntheses

Compounds **3**–**8** and all reagents were purchased from Sigma-Aldrich unless specified otherwise.

Compounds **1**, **2** were synthesized starting from 2,6-di-*tert*-butylphenol according previously described method with slight modifications (Mueller et al., 1961).

2.1.1. 3,5-di-tert-butyl-4-hydroxythiocyanate

To an ice cooled mixture of 10.31 g (0.05 mol) of 2,6-di-*tert*-butylphenol and 17.65 g (0.18 mol) KSCN in 75 mL of MeOH saturated with KBr 8.0 g of Br_2 (0.05 mol) in 20 mL MeOH saturated with KBr was slowly added drop by drop with constant stirring. Reaction mixture was stirred for 24 h at room temperature, and then 100 mL of water were added and extracted with petroleum ether (b.p. 40–70 °C). The extracts were dried overnight over Na_2SO_4, after filtration the solvent was removed in a vacuum. The remaining yellow crystalline mass was washed with small volume of petroleum ether and dried in vacuum. Yield 12.1 g (92% yield) in the form of light yellow crystals with M.p. 60–62 °C (M.p. 59–62 °C (Mueller et al., 1961)).

2.1.2. 2,6-di-tert-butyl-4-mercaptophenol (*1*)

To 13.16 g (0.05 mol) of 3,5-di-*tert*-butyl-4-hydroxythiocyanate and 6.5 g (0.25 mol) of Zn dust in 130 mL of EtOH in an argon atmosphere, 25 mL of conc. HCl were added drop by drop under heating and stirring. The mixture was boiled for 4 h, and then cooled to room temperature. The solid precipitate was filtered off, washed with water, dried in air for 24 h. The product was dissolved in CH_2Cl_2, unreacted Zn was filtered off, than the solvent was removed in vacuum. Yield 9.05 g (76.1%) of white powder. M.p. 82–84 °C (M.p. 84–85 °C (Mueller et al., 1961)).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.45 (s, 18H, 2 Bu^t); 3.38 (s, 1H, SH); 5.18 (s, 1H, 1 OH); 7.20 (s, 2H, C_6H_2); ^{13}C NMR (100.6 MHz, $CDCl_3$, δ, ppm): 30.16 (C($\underline{C}H_3)_3$); 34.37 ($\underline{C}(CH_3)_3$); 118.27 (C1, Ar); 127.34 (2C2 Ar); 137.00 (2C3 Ar); 152.89 (C4 Ar).

2.1.3. Bis(3,5-di-tert-butyl-4-hydroxyphenyl)disulfide (*2*)

A mixture of 2.63 g (0.01 mol) of 3,5-di-*tert*-butyl-4-hydroxythiocyanate in 10% water solution of KOH was heated in Ar atmosphere for 5 days, than the yellow disulfide was extracted by extracted with petroleum ether (b.p. 40–70 °C), washed and dried over Na_2SO_4. The solvent was was removed in a vacuum, and residue was recrystallized from petroleum ether (Sdp. 50-70″). Yield 1.23 g (52%), M.p. 144–145 °C (M.p. 145–146 °C (Mueller et al., 1961)).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.41 (s, 18H, 4 Bu^t); 5.28 (s, 2H, 2 OH); 7.34 (s, 4H, 2 C_6H_2); ^{13}C NMR (100.6 MHz, $CDCl_3$, δ, ppm): 29.73 (C($\underline{C}H_3)_3$); 34.06 ($\underline{C}(CH_3)_3$); 127.19 (C1, Ar); 127.36 (2C2 Ar); 136.27 (2C3 Ar); 153.78 (C4 Ar).

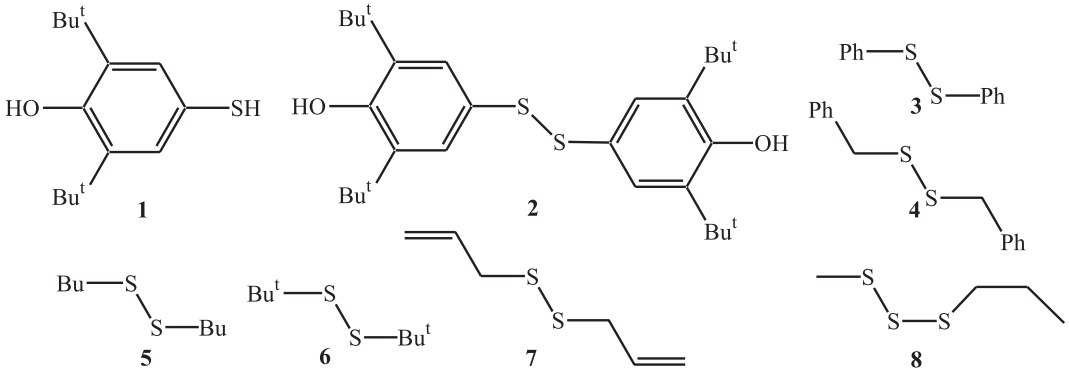

Fig. 1 Structures of organosulfur compounds **1**–**8**: 2,6-di-*tert*-butyl-4-mercaptophenol (**1**), bis(3,5-di-*tert*-butyl-4-hydroxyphenyl) disulfide (**2**), diphenyl disulfide (**3**), dibenzyl disulfide (**4**), dibutyl disulfide (**5**), di-*tert*-butyl disulfide (**6**), diallyl disulfide (**7**), methyl propyl trisulfide (**8**).

2.2. DPPH radical scavenging activity

All experiments were performed with a 96-cell "Zenyth 200RT, Anthos" microplate spectrophotometer. The free radical scavenging activity was evaluated using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to the method described by Brand-Williams et al. (1995) with a slight modification. For each test compound different concentrations in MeOH were used (20, 40, 80, 120, 160 and 200 μM). The stock DPPH solution contained 0.2 mM of radical in MeOH. 0.1 mL of the test compound solution was added to 0.1 mL of DPPH solution (0.2 mM) in each cell so that the initial DPPH concentration in cells was 0.1 mM. The microplate was placed in a spectrophotometer and the decrease in the absorbance values of DPPH solution for 40 min at 20 °C was measured at λ_{max} = 517 nm. The results were expressed as scavenging activity, calculated as follows:

$$\text{Scavenging activity, } \% = [(A_c - A_s)/A_c] \times 100$$

The concentration of the compound needed to decrease 50% of the initial DPPH concentration (EC_{50}) was determined to evaluate the antioxidant effect. The EC_{50} values were calculated graphically by plotting scavenging activity against compounds concentration.

2.3. Cupric reducing antioxidant capacity (CUPRAC assay)

Neocuproine (2,9-dimethyl-1,10-phenanthroline) and Trolox were used with no further purification. The method proposed by Apak et al. (2004) was used with slight modification. For these measurements, 0.05 mL of $CuCl_2$ solution (0.01 M), 0.05 mL of MeOH neocuproine solution (7.5 mM) and 0.05 mL of ammonium acetate buffer solution (1 M) were added to a test tube followed by mixing with the 0.05 mL tested compounds (0.5 mM). The mixtures were kept at room temperature for 30 min. All experiments were performed with a 96-cell "Zenyth 200RT, Anthos" microplate spectrophotometer, absorbance was measured at 450 nm against a reagent blank. The increase in absorbance of the reaction mixture in comparison with the control indicates the reduction capability of the test compound. The results were presented in Trolox equivalents (Trolox Equivalent Antioxidant Capacity, TEAC) obtained using absorbance data and the linear calibration curve plotted as absorbance *vs*. Trolox concentration.

2.4. Ferrous ions chelating activity (FIC)

The chelation of ferrous ions by compounds was estimated by method of Dinis et al. (1994). Briefly, 10 μl of 2 mM $FeCl_2$ was added to 20 μl of the investigated compound (5 mM) and 150 μl of EtOH. The reaction was initiated by the addition of 40 μl of 5 mM ferrozine solution. The mixture was left to stand at 35 °C for 10 min. The absorbance of the solution was thereafter measured at 562 nm.

The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated as:

$$\text{FIC}, \% = [(A_c - A_s)/A_c] \times 100$$

where A_c was the absorbance of the control, and A_s was the absorbance of the sample. Na_2EDTA was used as positive control.

2.5. Determination of LOOH and TBARS concentrations in oleic acid

The determination of oleic acid oxidation level was performed by the kinetic measuring of the total concentration of the corresponding isomeric LOOH using iodometric titration (Rzhavskaya, 1976). The oxidation of constant volume of the acid (15 mL) was carried out in a thermostatic cell using an air flow at 65 °C during 3 h. The oxidation proceeds in the conditions, the oxidation rate is independent of air volume passing through the cell (Emanuel, 1974).

The concentrations of the additives were 1 mM compared with the initial concentration of LOOH in the reaction mixture. Oleic acid (1 mL), $CHCl_3$ (12 mL), glacial AcOH (18 mL), and freshly prepared cold-saturated KI solution (1 mL) were placed in a flask and shaken for 2 min; distilled water (100 mL) and a 1% starch solution (1 mL) were then added, and the resulting mixture was immediately titrated with $Na_2S_2O_3$ solution (0.01 N). Iodine released in an amount equivalent to that of LOOH was titrated with a standard thiosulfate solution. At the same time, a control test for reagents was carried out: all the reagents except for oleic acid were added to the flask.

The LOOH concentration was calculated according to the following formula:

$$[\text{LOOH}] = [(V_s - V_c) \times 0.001269 \times K \times 100]/m,$$

wherein V_s is the volume of 0.01 N $Na_2S_2O_3$ solution, consumed during the titration of working sample, mL; V_c is the volume of 0.01 N $Na_2S_2O_3$ solution, consumed during the titration of control, mL; K is the conversion factor to the exactly 0.01 N $Na_2S_2O_3$ solution; m is the mass of studied oleic acid; and 0.001269 is the amount of I_2 expressed in g, equivalent to 1 mL of 0.01 N $Na_2S_2O_3$ solution. The [LOOH, mmol/L] content equal to 1% corresponds to 78.7 mM of active O_2 per 1 L of lipids.

The accumulation rate for the final oxidation products (TBARS) was determined according to a modified standard method (Stroev et al., 2012). The studied compounds (1 mM) were added to oleic acid thermostated at the selected temperature. Samples (0.01 mL) of oleic acid were taken from the thermostat every 30 min. They were introduced into a test tube containing a mixture of Tris buffer (0.8 mL), distilled water (1.2 mL), and freshly prepared thiobarbituric acid solution (0.8%, 1 mL); the tube was placed for 10 min in a boiling water bath, and after cooling, the absorption of the samples was measured in comparison with that of control at λ = 532 nm. A similar mixture, but without added oleic acid, was used as the control.

The concentration of carbonyl compounds was calculated according to the formula:

$$[TBARS] = (E \times 3)/0.156$$

where [TBARS] is the content of carbonyl compounds, nM; E is the extinction of a sample relative to the control; 3 is the sample volume, mL; and 0.156 is the extinction of malondialdehyde (1 nmol) dissolved in 1 mL at λ = 532 nm.

The kinetic curves of the oleic acid peroxidation in the presence of studied compounds follow the exponential law, the approximation coefficients of kinetic curves were in a range of 0.9031–0.9731. Kinetic investigation has shown the initials rate of the hydroperoxides LOOH accumulation to be

pseudo-first-order in the air. The kinetic curves of content of secondary carbonyl products TBARS during the oleic acid oxidation in the presence of compounds follow the linear law, the approximation coefficients of kinetic curves were in a range of 0.9024–0.9922.

2.6. Determination of TBARS accumulation in the Russian sturgeon liver homogenate

The experiments *in vitro* were carried out using the liver of a Russian sturgeon raised in Unique aqua complex for the reproduction of valuable fish species of the Federal Research Center Southern Scientific Center of the Russian Academy of Sciences within the framework of State Assignment (Project No. 01201354245). All manipulations were conducted according to the international rules GLP (Good Laboratory Practice). The samples of fish liver were fixed in the cold.

The LPO intensity was estimated according to the accumulation of carbonyl products forming a colored complex with thiobarbituric acid (TBARS) (Stroev et al., 2012). A liver of Russian sturgeon (10 g) homogenized in the cold and studied compounds were added at the concentration of 0.1 mM in $CHCl_3$ to a solution of potassium chloride precooled to 0–4 °C. The absence of any influence of $CHCl_3$ on the LPO rate in the control was preliminarily established under these conditions. The resulting mixture was poured into flasks and incubated with the added studied compounds at the temperature of 5 °C for 48 h, sampling the mixture (2 mL) at a certain time interval into tubes for a subsequent centrifugation. Solutions of ascorbic acid (0.1 mL), Mohr's salt (0.1 mL), and trichloroacetic acid (1 mL) were added to these tubes. The tubes were placed in a water bath at 37 °C for 10 min and then centrifuged for 10 min (3000 rpm). The supernatants (2 mL) were placed into clean tubes, thiobarbituric acid solutions (1 mL) were added, the samples were placed in a boiling water bath for 10 min, and then they were cooled in ice water down to room temperature (~20 °C). After cooling, $CHCl_3$ (1.0 mL) was added to the samples to give a clear solution, and the resulting mixtures were centrifuged for 15 min (3000 rpm). The supernatant was sampled, and the extinction of sample was measured using a SF-103 spectrophotometer at $\lambda = 532$ nm relative to the control sample. The calculation was carried out according to the formula:

$$X = (E \times 3 \times 3.2)/(0.156 \times 2),$$

wherein X is the content of carbonyl products in the starting homogenate, nmol; E is the extinction of samples; 3 is the volume of samples, mL; 3.2 is the total volume of studied samples, mL; 0.156 is the extinction of malondialdehyde (1 nmol) dissolved in 1 mL at $\lambda = 532$ nm; 2 is the volume of supernatant used to determine carbonyl products, mL.

The efficiency of the antioxidant action (EAA, %) of the compounds **1**–**8** (Antonova et al., 2008) was calculated from the relation

$$EAA = [(C_c - C_s)/C_c] \times 100\%,$$

where C_c and C_s are concentrations the carbonyl products forming a colored complex with thiobarbituric acid (TBARS) in the control and the sample of test liver homogenates Russian sturgeon, respectively. If the EAA is positive, the test compound shows an antioxidant action; a negative EAA value attests to a prooxidant action.

2.7. Statistical analysis

The acquired results were statistically processed using the Student *t*-test (implemented in Microsoft Excel software), and the average value and standard deviation were calculated. From five to ten repeats were carried out for the each experimental determination, and the nature of influence was estimated using the average values of activity, taking into account the experimental error ($p < 0.01$). Pearson's correlation coefficient (r) was determined to establish the relationship between the values of the parameters of antioxidant activity on various model systems *in vitro*.

3. Results and discussion

3.1. DPPH radical scavenging activity

The capacity to inhibit the development of radical chain reactions due to interaction with ROS and the formation of a corresponding highly stable radical is the main property of most known inhibitors of oxidative processes. One of the defining methods for revealing the antioxidant potential of compounds at the molecular level is the reaction with the stable 2,2-diphenyl-1-picrylhydrazyl radical DPPH (Gulcin et al., 2010).

The ability of compounds **1**–**8** to transfer a hydrogen atom to the stable DPPH radical with the formation of the corresponding radicals, which can then enter into reactions of recombination, dimerization and fragmentation, was investigated. Antiradical activity was measured by a colour change from violet to yellow and a decrease in absorbance at 517 nm. The amount of antioxidant required to reduce the initial DPPH concentration by 50% (half maximal inhibitory concentration (IC_{50})) was calculated.

It is known that there are two main mechanisms of the antiradical action of antioxidants: hydrogen atom transfer (HAT) and single electron transfer (SET). Despite the difference in mechanisms the same end products are formed (Esfandi et al., 2019). These processes can proceed in parallel. Although, depending on the structure of the antioxidant and conditions, one of the mechanisms can dominate. According to the results obtained, it was found that only organosulfur compounds with phenolic and/or *HS*-groups **1** and **2** demonstrated a pronounced radical scavenging activity (IC_{50} values were 68.01 ± 0.09 and 20.09 ± 0.02 μM, respectively) (Fig. 2), that indicates that these compounds act mainly through the HAT mechanism.

This is consistent with literature data, according to which sterically hindered phenols are good antioxidants due to their ability to form stable aroxyl radicals (Leopoldini et al., 2004; Amić et al., 2020). The presence of two similar fragments in compound **2** promoted the increase in antioxidant properties in comparison with diphenyl disulfide and compound **1**. Earlier, it was also found that sulfur-containing amino acids cysteine, methionine, taurine have different activities against DPPH (Kim et al., 2020), among them the highest one was explored for cysteine containing *HS*-group. On the contrary, the organic oligosulfides **3**–**8** do not exhibit antiradical activity in this reaction, but one must take into account that this

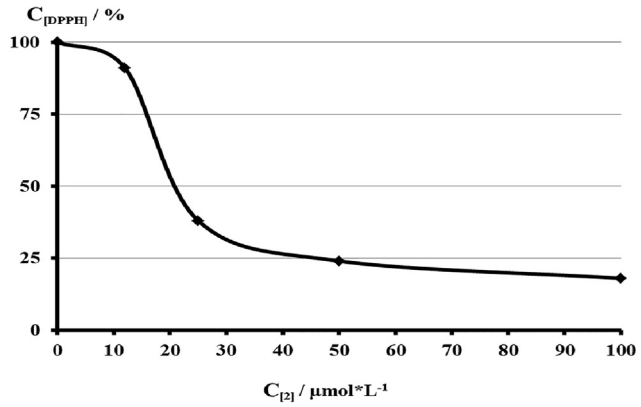

Fig. 2 Dependence of the amount (%) of unreduced DPPH on the concentration of bis-(4-hydroxy-2,6-di-*tert*-butylphenyl) disulfide **2** in MeOH solution.

method is not universal, since not all antioxidants can interact react quickly with active radicals like DPPH, and, therefore, it is necessary to study the antioxidant activity of such compounds by other test systems.

3.2. Cupric reducing antioxidant capacity (CUPRAC assay)

The CUPRAC assay is based on the ability of a potential antioxidant to act as an electron donor and reduce Cu^{2+} ion to Cu^{+} in combination with 2,9-dimethyl-1,10-phenanthroline (neocuproine). The absorbance of the solution compounds **1**–**8** were measured at 450 nm at room temperature. The calibration curves of each potential antioxidant were constructed as Trolox Equivalent Antioxidant Capacity ($TEAC_{CUPRAC}$), found as the ratio of the molar absorptivity of each compound to that of trolox were calculated (Dontha, 2016).

It was found that compound **2** in reducing ability was twice as active as trolox, and compound **4** exhibited activity similar to that of trolox despite the absence of a phenolic fragment in the structure, $TEAC_{CUPRAC}$ = 1.06 (Table 1).

The rest of the organosulfur compounds did not show high reducibility in this test system. It is important to note that compound **1**, despite the presence of *HO*- and *HS*-groups, also does not show a pronounced ability to act as an electron donor, reducing the copper ion in a complex with 2,9-dime thyl-1,10-phenanthroline.

3.3. Fe^{2+}-chelating activity compounds (FIC)

An independent type of pharmacological and antioxidant activity of compounds is the ability of them to act as a chelator of transition metals that catalyze the decomposition reactions of LOOH with the formation of ROS, in particular, a hydroxyl radical HO·. Antioxidants such as trolox, BHA and BHT can prevent the development of ferroptosis (Cao and Dixon, 2016). Absorption with food is considered among the main factors affecting the bioavailability of micronutrients for humans. Compounds with chelating activity enhance the absorption of calcium and iron ions in the digestive tract. It was shown that hydrolyzed proteins and peptides from food proteins can act as ligands of transition metals, inhibiting iron-dependent lipid peroxidation (Walters et al., 2018). Moreover, cysteine and methylcysteine can be used for decreasing of Fe (III) concentration in biological systems that helps to stop the development of oxidative stress (Tewari, 2002).

The Fe^{2+}-chelating activity of compounds **1**–**8** was studied spectrophotometrically based on the ability to bind Fe^{2+} ions, reducing the content of the coloured Fe^{2+}-ferrosine complex (λ = 562 nm). A pronounced Fe^{2+}-chelating activity was demonstrated for di-*tert*-butyl disulfide (**6**) and methyl propyl trisulfide (**8**) (>95%) (Table 1).

For the rest of the compounds, the Fe^{2+}-chelating activity is significantly lower than that of the standard EDTA, even in the case of compounds **1** and **2** with *HO*- and *HS*-groups in the structure. In this model system, the dependence of the Fe^{2+}-chelating activity on the nature of the organic radical (aliphatic, aromatic) and the number of sulfur atoms in the structure of the compounds has not been established.

3.4. Influence of sulfur-containing compounds on the oxidation of cis-9-octadecenoic acid

Increased formation of primary and secondary products of lipid peroxidation (LPO) is a marker of the development of oxidative stress and is considered a universal pathogenetic factor that is responsible for the formation and development of a wide range of diseases and pathologies (Tsai and Huang, 2015; Krzystek-Korpacka et al., 2008). The oxidation of unsaturated fatty acids serves as a model reaction for the lipid peroxidation in cellular membranes bilayer. The oxidation of substrate (oleic acid) by molecular oxygen involves the generation of substituted radicals that interact with O_2 to produce peroxyl radicals LOO·. The rate of LOO· transformation to

Table 1 Antioxidant activity of compounds **1**–**8.**

Compound	$TEAC_{CUPRAC}$	FIC, %	LOOH, k_0/k_1	TBARS, % inhibition
1	0.74 ± 0.04	19.8 ± 0.8	0.77 ± 0.08	24.1 ± 0.32
2	2.04 ± 0.05	28.0 ± 0.3	0.49 ± 0.05	73.1 ± 1.30
3	0.10 ± 0.01	73.7 ± 1.5	0.79 ± 0.11	24.6 ± 0.29
4	1.06 ± 0.04	31.7 ± 0.8	0.89 ± 0.09	3.4 ± 0.06
5	0.07 ± 0.01	56.4 ± 0.9	0.95 ± 0.14	−9.2 ± 0.01
6	0.09 ± 0.01	95.8 ± 2.3	0.83 ± 0.04	32.3 ± 0.46
7	0.14 ± 0.03	22.7 ± 1.1	0.88 ± 0.03	10.6 ± 0.11
8	0.11 ± 0.02	95.3 ± 2.8	0.77 ± 0.07	20.7 ± 0.25

corresponding *cis*- and *trans*-isomeric hydroperoxides (LOOH) which are the main products of the initial reaction period (Porter et al., 1994) might be used as a criterion of the rate of the peroxidation (Frankel, 1980). The markers of carbonyl compounds formation following the LOOH decomposition are thiobarbituric acid reactive substances (TBARS), the accumulation of which in the presence of investigated compounds have been studied by UV–Vis spectrophotometry (λ_{max} = 532 nm).

On a model system for the oxidation of oleic acid with atmospheric oxygen at 65 °C for 3 h, the total antioxidant activity of organosulfur compounds was assessed by standard methods for the accumulation of LOOH and carbonyl products reacting with thiobarbituric acid.

The kinetic curves of LOOH accumulation in oleic acid are exponential and correspond to the equation $C = a \cdot e^{kt} + b$ with correlation coefficients close to 1, which indicates a pseudo-first order of reaction in the substrate, inherent in a radical process with degenerate chain branching (Figs. 3, 4).

The effect of sulfur-containing compounds show that decrease the amount of oleic acid hydroperoxides formed, except for compound **5**, the addition of which does not influence the level of oleic acid LOOH, the kinetic curves of oleic acid's autooxidation and in the presence of compound **5** are identical (Fig. 4).

The total content of LOOH after 3 h in the presence of sulfur-containing compounds when compared with oleic acid without additives amount 64–87%. The influence of compound **2** is more significant and the total concentration of LOOH is 53% lower than in the oleic acid autooxidation. The relative rate constants of the LOOH formation are given in Table 1 and the comparison of the k_0/k_1 values for the LOOH accumulation in the presence of the compounds **1**–**8** only shows inhibitory effect.

The content of TBARS during the oleic acid oxidation is also appropriately increasing over time without and with additive the sulfur-containing compounds **1**–**8**, the secondary carbonyl products accumulation curves are linear, corresponding to the equation [TBARS] = kt + b, the correlation coefficients are close to 1 (Figs. 5 and 6). However, the data confirm the inhibitory effect of sulfur-containing compounds, except for the compound **5** showing insignificant prooxidant activity (Fig. 6).

The level of TBARS after 3 h decreases in the presence of added compounds **1**, **3**, **6, 7** and **8** by 11–32% relative to the control (Table 1). The level of LPO in oleic acid after 3 h of incubation with of compound **4** does not differ from the control. Compound **5** exhibits a prooxidant effect increasing the level of peroxidation by 9%. The addition of compound **2** leads to a greater decrease 73% in the level of secondary carbonyl compounds (Fig. 5, Table 1). Interestingly, dibutyl disulfide **5** exhibited insignificant promoting effect in contrast to di-*tert*-butyl disulfide **6**.

It is known that organosulfur compounds exhibit antioxidant activity due to the ability to degrade LOOL and LOOH without the formation of active radicals that initiate chain radical processes leading to the development of oxidative stress

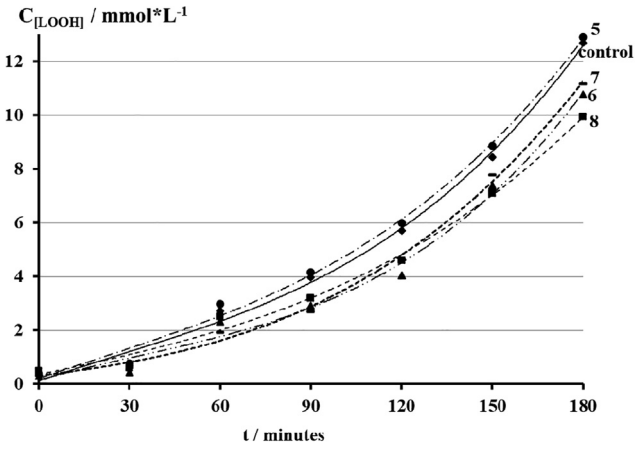

Fig. 4 Kinetic curves of LOOH formation in the presence of 1 mmol/L additives at 65 °C: (control) oleic acid without additives; (**5**) dibutyl disulfide; (**6**) di-*tert*-butyl disulfide; (**7**) diallyl disulfide; (**8**) methyl propyl trisulfide.

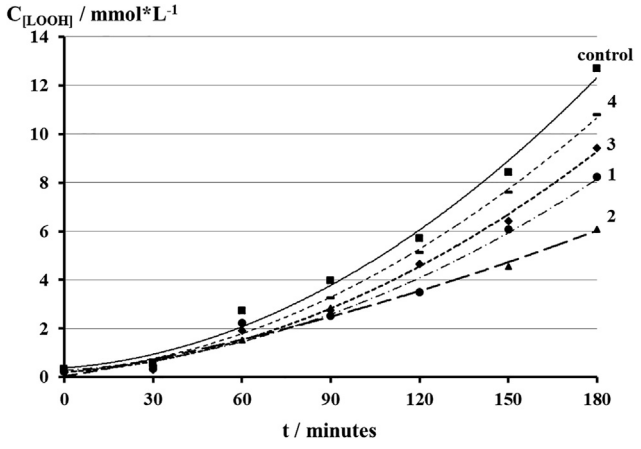

Fig. 3 Kinetic curves of LOOH formation in the presence of 1 mmol/L of different compounds at 65 °C: (control) oleic acid without additives; (**1**) 2,6-di-*tert*-butyl-4-mercaptophenol; (**2**) bis (3,5-di-*tert*-butyl-4-hydroxyphenyl) disulfide; (**3**) diphenyl disulfide; (**4**) dibenzyl disulfide.

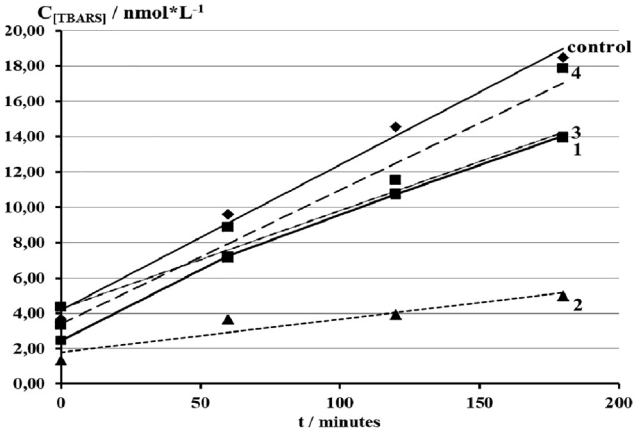

Fig. 5 Kinetic curves for the accumulation of TBARS in oleic acid in the presence of 1 mmol/L additives at 65 °C: (control) oleic acid without additives; (**1**) 2,6-di-*tert*-butyl-4-mercaptophenol; (**2**) bis(3,5-di-*tert*-butyl-4-hydroxyphenyl) disulfide; (**3**) diphenyl disulfide; (**4**) dibenzyl disulfide.

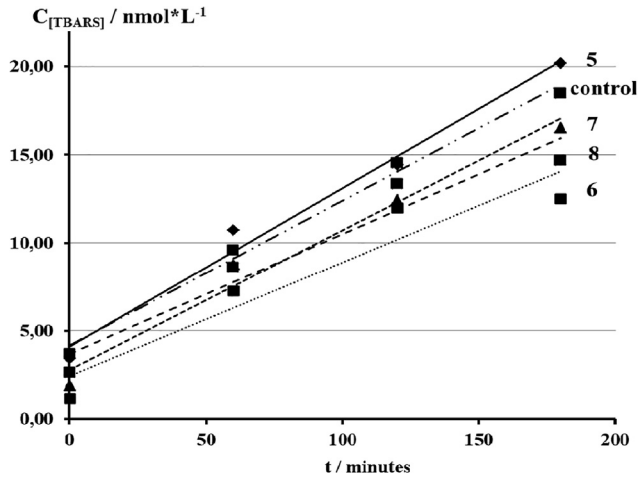

Fig. 6 Kinetic curves for the accumulation of TBARS in oleic acid in the presence of 1 mmol/L additives at 65 °C: (control) oleic acid without additives; (**5**) dibutyl disulfide; (**6**) di-*tert*-butyl disulfide; (**7**) diallyl disulfide; (**8**) methyl propyl trisulfide.

(Chauvin et al., 2019). At the same time, organosulfur compounds can act as free radical scavenger, for example, as in the case of di-*tert*-butyl sulfide, the oxidation of which forms the corresponding sulfoxide. Chauvin et al. (2019) also found that the reactivity of polysulfides is preserved even at elevated temperatures, which allows them to surpass phenols and alkylated diphenylamines in antiradical activity, which are widely used in medicine as antioxidants.

Sinse compounds **1**–**4**, **6**–**8** were active against both primary and secondary LPO products, these organosulfur compounds possess an intramolecular synergistic antioxidant effect. It was previously shown in various *in vitro* model systems that bis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl)sulfide (thiophane) is an effective polyfunctional antioxidant due to synergistic combination of two phenol pendants and antiperoxidase bivalent sulfur activity (Prosenko et al., 2003).

We investigated also the impact of sulfur-containing compounds on oleic acid peroxidation and the inhibitory effect of compound **2** has been proven (Figs. 3 and 5) that is associated with the key role of 2,6-di-*tert*-butylphenol moieties and two atoms of sulfur in molecule. The data presented do not demonstration the significant influence of the organic group nature and amount of sulfur atoms upon the effectiveness of compounds **1**–**8**.

3.5. Determination of TBARS level in the Russian sturgeon liver homogenate

The antioxidant potential of sulphur derivatives was studied using a model system of lipid peroxidation in sturgeon liver. The choice of the substrate is explained by the fact that liver cells are especially sensitive to any stressful conditions and can instantly respond, increasing the level of primary and secondary LPO products, as a result of which the process of apoptosis is activated, energy supply and detoxification mechanisms are disrupted. Oxidation of liver lipids which make up cell membranes directly causes damage to cells *in vivo* therefore this model system is similar to biological systems (Somerharju et al., 1999).

The inhibitory effect of organosulfur derivatives was studied under the conditions of a long-term LPO process, which made it possible to evaluate the effect of compounds under long-term oxidative stress, when the peroxidation process is enhanced, and the concentration of antioxidants is significantly consumed, and to reveal a possible inversion of anti-/prooxidant properties *in vitro* (Rao et al., 2011; Hořejší, 2005). The level of accumulation of secondary carbonyl products of LPO, forming colored complexes with thiobarbituric acid (TBARS), under autooxidation conditions and in the presence of the compounds under study is presented in Table 2. At subsequent stages of LPO, the activity of compound **1** is reduced to 15%, in contrast to compound **2**, for which an increase in the effectiveness of the antioxidant action (EAA) is noted up to 64% (Fig. 7).

Aliphatic symmetric disulfides **5**–**7** showed efficiency only at the initial (60 min) and middle (1441 min) stages of LPO, at the last stage (2880 min) a significant decrease in antioxidant activity was established, the level of TBARS is comparable to the control, but no inversion is observed.

Table 2 Influence of sulfur organic compounds and BHT on the amount of TBARS in the liver homogenate of Russian sturgeon during long-term *in vitro* oxidation.

Compounds	TBARS, nmol/g		
	1 h	**24 h**	**48 h**
control	16.85 ± 0.10	24.21 ± 0.38	31.61 ± 0.22
1	8.76 ± 0.23	15.98 ± 0.26	27.00 ± 0.33
2	10.95 ± 0.28	10.17 ± 0.19	11.42 ± 0.24
3	20.73 ± 0.14	27.60 ± 0.22	39.11 ± 0.21
4	14.66 ± 0.31	22.27 ± 0.12	32.53 ± 0.11
5	12.81 ± 0.29	16.46 ± 0.37	30.80 ± 0.17
6	12.64 ± 0.32	17.43 ± 0.36	28.73 ± 0.38
7	12.97 ± 0.15	18.40 ± 0.29	30.46 ± 0.34
8	13.82 ± 0.22	22.03 ± 0.29	31.84 ± 0.41

Average values for the series of experiments are given; differences from the control experiment group: $p < 0.01$.

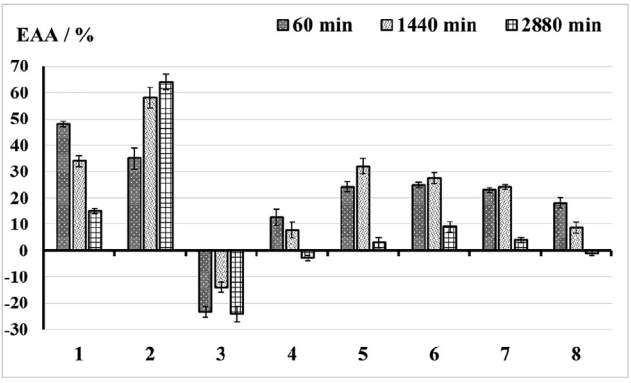

Fig. 7 The efficiency of the antioxidant action (EAA, %) of the compounds **1**–**8** during long-term *in vitro* oxidation of Russian sturgeon liver lipids: (**1**) 2,6-di-*tert*-butyl-4-mercaptophenol; (**2**) bis(3,5-di-*tert*-butyl-4-hydroxyphenyl) disulfide; (**3**) diphenyl disulfide; (**4**) dibenzyl disulfide; (**5**) dibutyl disulfide; (**6**) di-*tert*-butyl disulfide; (**7**) diallyl disulfide; (**8**) methyl propyl trisulfide.

Table 3 Pearson's correlation coefficient between the values of the parameters of antioxidant activity on various model systems *in vitro*.

	DPPH, % of control	$TEAC_{CUPRAC}$	FIC, %	LOOH oleic acid, % of inhibition	TBARS oleic acid, % of inhibition
$TEAC_{CUPRAC}$	0.2125				
FIC, %	−0.3071	−0.5746			
LOOH oleic acid, % of inhibition	0.1429	0.7636	−0.1749		
TBARS oleic acid, % of inhibition	−0.1290	0.6807	−0.0510	0.9179	
EAA, %	−0.3308	0.8101	−0.4001	0.6701	0.7935

It has previously been found that diallyl disulfide **7** can prevent acetaminophen-induced nephrotoxicity through its antioxidant properties (Ko et al., 2017).

It has also been shown in experimental rats with myocardial dysfunction that supplementation of garlic oil containing diallyl disulfide significantly reduced TBARS levels, cardiac lactate dehydrogenase activity, and increased endogenous antioxidant levels (Asdaq and Avula, 2015). At the same time, it was found that an essential oil extract from white cabbage containing dimethyltrisulfide and dimethyldisulfide are more potent inhibitors of LPO than diallyl disulfide, which explains its hepatoprotective effect (Morales-López et al., 2017).

Insignificant antioxidant activity is noted at all stages of oxidative destruction of sturgeon liver lipids dibenzyl disulfide **4** and methylpropyl trisulfide **8**, and compound **3** exhibits prolonged prooxidant activity. Compound **2** exhibits a pronounced antioxidant effect at all stages, as in other model systems. The experimental results evidence that compound **2** acts as an effective inhibitor in this model system too, what is explained the hydrogen atom abstraction from the phenol fragments in compound **2** gives relatively stable radical.

3.6. Pearson's correlation analysis

To assess the relationship between various indicators of the antioxidant activity of organosulfur compounds **1**–**8**, Pearson's correlation analysis was carried out (Lesaffre et al., 2009) and the calculated coefficients are presented in Table 3.

Significant positive correlations were observed between the accumulation rate of LOOH and TBARS in oleic acid ($r = 0.9179$). A moderate positive correlation was found between the reducing activity of CUPRAC and LOOH and TBARS in oleic acid and EAA in sturgeon liver homogenate ($r = 0.7636$, 0.6807 and 0.8101, respectively), as well as between the effectiveness of the antioxidant action and the level of accumulation of primary and secondary products of oleic acid oxidation ($r = 0.6701$ and 0.7935).

The worst negative correlations were noted between iron chelating activity (FIC), reductive ability in CUPRAC and inhibition of the process of long-term LPO in Russian sturgeon liver (EAA,%) ($r = -0.5746$ and −0.4001, respectively).

4. Conclusion

In order to estimate the pharmacological perspectives of a series of organosulfur derivatives the comparative study of antioxidant action of synthetic compounds **1** and **2** containing 2,6-di-*tert*-butylphenol moiety with organosulfur compounds **3**–**8** that occur in medicinal plant by various model assays (DPPH-, CUPRAC tests, iron chelating ability (FIC-assay), model reaction of unsaturated organic acid and lipid peroxidation *in vitro*) was performed. The high antioxidant activity of synthetic analogs **1** and **2** compared to **3**–**8** was found. The pronounced activity of bis(3,5-di-*tert*-butyl-4-hydroxyphenyl) disulfide **2** in all model systems is explained by the intramolecular synergism of the antioxidant actions owing to the presence of two radical scavenging 2,6-di-*tert*-butylphenol fragments and an antiperoxide disulfide group for which the possibility of homolytic cleavage of the *S*–*S* bond and formation of stable perthiyl and thiyl radicals inhibiting the development of radical chain reactions. The prooxidant activity of compound **5** in oleic acid peroxidation and compound **3** in long-term oxidation of sturgeon liver lipids was revealed. The high efficiency of the antioxidant action the synthetic analogs of natural organosulfur compounds **1** and **2** allows considering this compounds for practical use as an effective inhibitors of oxidative processes. The high antioxidant efficiency let one to consider compound **2** for practical use as an effective inhibitor of peroxidation in biological substrates and cytoprotector.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the Russian Science Foundation (grant № 20-13-00084, investigation of antioxidant activity) and Russian Foundation for Basic Research (grant № 20-03-00471, synthesis of compounds).

References

Adewole, K.E., 2020. Nigerian antimalarial plants and their anticancer potential: A review. J. Integr. Med. 18 (2), 92–113. https://doi.org/10.1016/j.joim.2020.01.001.

Amić, A., Marković, Z., Dimitrić Marković, J.M., Milenković, D., Stepanić, V., 2020. Antioxidative potential of ferulic acid phenoxyl radical. Phytochemistry 170, 112218. https://doi.org/10.1016/j.phytochem.2019.112218.

Antonova, N.A., Kolyada, M.N., Osipova, V.P., Pimenov, Y.T., Berberova, N.T., Tyurin, V.Y., Gracheva, Y.A., Milaeva, E.R., 2008. Study of the antioxidant properties of phosphorylated phenols. Dokl. Chem. 419 (1), 62–64. https://doi.org/10.1134/S0012500808030051.

Anyasor, G.N., Ogunbiyi, B., Akinlusi, I.A., 2017. Antioxidant and anti-inflammatory properties of selected polyherbal preparations: Oroki herbal, Swedishbitters and Yoyo bitters. Oxidants Antioxidants Med. Sci. 6 (2), 25–29. https://doi.org/10.5455/oams.190417.or.106.

Apak, R., Guglu, K., Ozyurek, M., Karademir, S., 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J. Agric. Food. Chem. 52 (26), 7970–7981. https://doi.org/10.1021/jf048741x.

Asdaq, S.M.B., Avula, P.R., 2015. Interaction of garlic oil and its constituent diallyl disulfide with atenolol in isoproterenol induced acute myocardial injury in rats. J. Pharmaceut. Biol. 5 (1), 44–52.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol.. 28 (1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Cao, J.Y., Dixon, S.J., 2016. Mechanisms of ferroptosis. Cell. Mol. Life Sci. 73, 2195–2209. https://doi.org/10.1007/s00018-016-2194-1.

Cerella, C., Dicato, M., Jacob, C., Diederich, M., 2011. Chemical properties and mechanisms determining the anti-cancer action of garlic-derived organic sulfur compounds. Anti-Cancer Agents Med. Chem. 11, 3, 267–271. https://doi.org/10.2174/187152011795347522.

Chauvin, J.-P., Griesser, M., Pratt, D.A., 2019. The antioxidant activity of polysulfides: it's radical!. Chem. Sci. 19, 4999–5010. https://doi.org/10.1039/C9SC00276F.

Colín-González, A.L., Santamaría, A., 2017. Garlic, gastrointestinal protection and oxidative stress. Gastrointestinal Tissue, 275–288. https://doi.org/10.1016/b978-0-12-805377-5.00020-5.

Delgado-Marín, L., Sánchez-Borzone, M., García, D.A., 2017. Neuroprotective effects of gabaergic phenols correlated with their pharmacological and antioxidant properties. Life Sci. 175, 11–15. https://doi.org/10.1016/j.lfs.2017.03.005.

Denisov, E., 1995. Handbook of Antioxidants, CRC Press, Boca Raton, FL. https://doi.org/10.1201/9781351072373.

Denisov, E, Afanas'ev, I. 2005. Oxidation and Antioxidants in Organic Chemistry and Biology. CRC Press, New York, USA.

Dinis, T.C.P., Madeira, V.M.C., Almeida, L.M., 1994. Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315, 161–169. https://doi.org/10.1006/abbi.1994.1485.

Diniyah, N., Alam, Md.B., Lee, S.-H., 2020. Antioxidant potential of non-oil seed legumes of Indonesian's ethnobotanical extracts. Arabian J. Chem. 13 (5), 5208–5217. https://doi.org/10.1016/j.arabjc.2020.02.019.

Dontha, S., 2016. A review on antioxidant methods. Asian J. Pharm. Clin. Res. 9 (8), 14–32. https://doi.org/10.22159/ajpcr.2016.v9s2.13092.

Emanuel, N.M., 1974. Kinetics and mechanism of chain reactions of liquid-phase oxidation of hydrocarbons. Bull. Acad. Sci. USSR, Division Chem. Sci. 23, 1010–1023. https://doi.org/10.1007/BF00927276.

Esfandi, R., Walters, M.E., Tsopmo, A., 2019. Antioxidant properties and potential mechanisms of hydrolyzed proteins and peptides from cereals. Heliyon. 5, e01538. https://doi.org/10.1016/j.heliyon.2019.e01538.

Final Report on the Safety Assessment of BHT, 2002. Int. J. Toxicol. 21(2), 19–94. https://doi.org/10.1080/10915810290096513.

Finkel, T., 2005. Radical medicine: Treating ageing to cure disease. Nat. Rev. Mol. Cell Biol. 6 (1), 971–976. https://doi.org/10.1038/nrm1763.

Gilgun-Sherki, Y., Rosenbaum, Z., Melamed, E., Offen, D., 2002. Antioxidant therapy in acute central nervous system injury: current state. Pharmacol. Rev. 54 (2), 271–284.

Ndoye Foe, F.M., Tchinang, T.F.K., Nyegue, A.M., Abdou, J.-P., Yaya, A.J.G., Tchinda, A.T., Essame, J.-L.O., Etoa, F.-X., 2016. Chemical composition, *in vitro* antioxidant and anti-inflammatory properties of essential oils of four dietary and medicinal plants from Cameroon. BMC Complement. Alternative Med. 16, 117. https://doi.org/10.1186/s12906-016-1096-y.

Frankel, E.N., 1980. Analytical methods used in the study of autoxidation processes. In: Simic, M.G., Karel, M. (Eds.), Autoxidation in Food and Biological Systems. Springer, Boston, MA, pp. 141–170. https://doi.org/10.1007/978-1-4757-9351-2_9.

Green, A.R., Shuaib, A., 2006. Therapeutic strategies for the treatment of stroke. Drug Discov Today. 11 (15–16), 681–693. https://doi.org/10.1016/j.drudis.2006.06.001.

Gulcin, I., Kireçci, E., Akkemik, E., Topal, F., Hisar, O., 2010. Antioxidant, antibacterial, and anticandidal activities of an aquatic plant: duckweed (Lemna minor L. Lemnaceae). Turkish J. Biol. 34, 175–188. https://doi.org/10.3906/biy-0806-7.

Hořejší, V., 2005. Lipid rafts and their roles in T-cell activation. Microbes Infect. 7 (2), 310–316. https://doi.org/10.1016/j.micinf.2004.12.004.

Kahl, R., Kappus, H., 1993. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Z Lebensm Unters Forsch. 196 (4), 329–338. https://doi.org/10.1007/BF01197931.

Kimura, H., 2015. Signaling molecules: hydrogen sulfide and polysulfide. Antioxid. Redox Signal. 22, 5, 362–376. https://doi.org/10.1089/ars.2014.5869.

Kim, J.-H., Jang, H.-J., Cho, W.-Y., Yeon, S.-J., Lee, Ch.-H., 2020. *In vitro* antioxidant actions of sulfur-containing amino acids. Arabian J. Chem. 13, 1678–1684. https://doi.org/10.1016/j.arabjc.2017.12.036.

Klein, J.A., Ackerman, S.L., 2003. Oxidative stress, cell cycle, and neurodegeneration. J. Clin. Investig. 111 (6), 785–793. https://doi.org/10.1172/JCI18182.

Ko, J.-W., Shin, J.-Y., Kim, J.-W., Park, S.-H., Shin, N.-R., Lee, I.-C., Shin, I.-S., Moon, C., Kim, S.-H., Kim, S.-H., Kim, J.-C., 2017. Protective effects of diallyl disulfide against acetaminophen-induced nephrotoxicity: A possible role of CYP2E1 and NF-κB. Food Chem. Toxicol. 102, 156–165. https://doi.org/10.1016/j.fct.2017.02.021.

Krzystek-Korpacka, M., Salmonowicz, B., Boehm, D., Berdowska, I., Zielinski, B., Patryn, E., Noczynska, A., Gamian, A., 2008. Diagnostic potential of oxidative stress markers in children and adolescents with type 1 diabetes. Clin. Biochem. 41 (1–2), 48–55. https://doi.org/10.1016/j.clinbiochem.2007.10.003.

Lee, J., Koo, N., Min, D.B., 2004. Reactive oxygen species, aging, and antioxidative nutraceuticals. Compr. Rev. Food Sci. Food Saf. 3 (1), 21–33. https://doi.org/10.1111/j.1541-4337.2004.tb00058.x.

Leopoldini, M., Marino, T., Russo, N., Toscano, M., 2004. Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. J. Phys. Chem. A. 108 (22), 4916–4922. https://doi.org/10.1021/jp037247d.

Lesaffre, E., Feine, J., Leroux, B., Declerck, D., 2009. Statistical and Methodological Aspects of Oral Health Research. John Wiley & Sons Ltd, Cornwall.

Li, S., Tan, H.Y., Wang, N., Zhang, Z.J., Lao, L., Wong, C.W., Feng, Y., 2015. The role of oxidative stress and antioxidants in liver diseases. Int. J. Mol. Sci. 16 (11), 26087–26124. https://doi.org/10.3390/ijms161125942.

Martins, N., Petropoulos, S., Ferreira, I.C.F.R., 2016. Chemical composition and bioactive compounds of garlic (Allium sativumL.) as affected by pre- and post-harvest conditions: A review. Food Chem. 211, 41–50. https://doi.org/10.1016/j.foodchem.2016.05.029.

Mahmoudi, R., Ehsani, A., Tajik, H., Pajohi-Alamoti, M., 2013. Evaluation of phytochemical and antibacterial properties of some medicinal plants from Iran. J. Biol. Active Products Nat. 3 (5–6), 310–322.

Milaeva, E.R., Shpakovsky, D.B., Gracheva, Yu.A., Antonenko T.A., Ksenofontova, T.D., Nikitin, E.A., Berseneva A.D., Novel selec-

tive anticancer agents based on Sn and Au complexes. Mini-review, 2020. Pure Appl. Chem. 92, 8, 1201–1216. https://doi.org/10.1515/pac-2019-1209

Morales-López, J., Centeno-Álvarez, M., Nieto-Camacho, A., López, M.G., Pérez-Hernández, E., Pérez-Hernández, N., Fernández-Martínez, E., 2017. Evaluation of antioxidant and hepatoprotective effects of white cabbage essential oil. Pharm. Biol. 55 (1), 233–241. https://doi.org/10.1080/13880209.2016.1258424.

Mueller, E., Stegmann, H.B., Scheffler, K., 1961. Ober Sauerstof-fradikale, XXI Untersuchungen an schwefelhaltigenar oxylen Mittels der elektronenresonanz. Annalen der Chemie, Justus Liebigs 645, 79–91.

Munné-Bosch, S., Pintó-Marijuan, M., 2017. Free Radicals, Oxidative Stress and Antioxidants. Reference Module Life Sci. Encyclopedia Appl. Plant Sci. (Second Edition). 1, 16–19. https://doi.org/10.1016/B978-0-12-394807-6.00077-0.

Nicastro, H.L., Ross, S.A., Milner, J.A., 2015. Garlic and onions: their cancer prevention properties. Cancer Prevent. Res. 8 (3), 181–189. https://doi.org/10.1158/1940-6207.CAPR-14-0172.

Osipova, V., Polovinkina, M., Osipova, A., Gracheva, Yu., Okhlobystin, A., 2019. *In silico* and *in vitro* evaluation of biological activity of some organic sulfur-containing compounds. Turk. J. Chem. 43, 1336–1349. https://doi.org/10.3906/kim-1904-40.

Parsaeimehr, A., Iqbal, H.M.N., Parra-Saldívar, R., 2018. Chapter 6 - Natural Products as Immune System Modulators, and Against Infections of the Central Nervous System, Clinical Microbiology: Diagnosis, Treatments and Prophylaxis of Infections, The Microbiology of Central Nervous System Infections, Academic Press, Volume 3, pp. 99–119, https://doi.org/10.1016/B978-0-12-813806-9.00006-8

Petrovic, V., Nepal, A., Olaisen, C., Bachke, S., Hira, J., Søgaard, C. K., Røst, L.M., Misund, K., Andreassen, T., Melø, T.M., Bartsova, Z., Bruheim, P., Otterlei, M., 2018. Anti-cancer potential of homemade fresh garlic extract is related to increased endoplasmic reticulum stress. Nutrients, 10(4), E450. doi: 10.3390/nu10040450.

Pham-Huy, L. Ai., He, H., Pham-Huy, Ch., 2008. Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci. 4 (2), 89–96.

Porter, N.A., Mills, K.A., Carter, R.L., 1994. A mechanistic study of oleate autoxidation: competing peroxyl H-atom abstraction and rearrangement. J. Am. Chem. Soc. 116 (15), 6690–6696. https://doi.org/10.1021/ja00094a026.

Pradeep, S.R., Srinivasan, K., 2017. Amelioration of hyperglycemia and associated metabolic abnormalities by a combination of fenugreek (Trigonella foenum-graecum) seeds and onion (Allium cepa) in experimental diabetes. J. Basic Clin. Physiol. Pharmacol. 28 (5), 493–505. https://doi.org/10.1515/jbcpp-2015-0140.

Prosenko, A.E., Terakh, E.I., Gorokh, E.A., Nikulina, V.V., Grigorév, I.A., 2003. Synthesis and antioxidant properties of bis[3-(3,5-dialkyl-4-hydroxyphenyl)alkyl] sulfides. Russ. J. Appl. Chem. 76 (2), 248–252. https://doi.org/10.1023/A:1024650612317.

Rao, P.S., Kalva, S., Yerramilli, A., Mamidi, S., 2011. Free radicals and tissue damage: role of antioxidants. Free Radicals and Antioxidants 1(4), 2–7. https://doi.org/10.5530/ax.2011.4.2.

Ryu, J.H., Kang, D., 2017. Physicochemical properties, biological activity, health benefits, and general limitations of aged black garlic: a review. Molecules 22 (6), 919. https://doi.org/10.3390/molecules22060919.

Rzhavskaya, F.M., 1976. Zhiry ryb i morskikh mlekopitayushchikh [Fats of Fish and Marine Mammals], Pitshevaya prom-t', Moscow.

Somerharju, P., Virtanen, J.A., Cheng, K.H., 1999. Lateral organisation of membrane lipids. The superlattice view. Biochim. Biophys. Acta 1440 (1), 32–48. https://doi.org/10.1016/s1388-1981(99)00106-7.

Stavrovskaya, I.G., Kristal, B.S., 2005. The powerhouse takes control of the cell: is the mitochondrial permeability transition a viable therapeutic target against neuronal dysfunction and death? Free Radical. Biol. Med. 38 (6), 687–697. https://doi.org/10.1016/j.freeradbiomed.2004.11.032.

Stefanucci, A., Zengin, G., Llorent-Martinez, E.J., Dimmito, M.P., Della Valle, A., Pieretti, S., Ak, G., Sinan, K.I., Mollica, A., 2020. Chemical characterization, antioxidant properties and enzyme inhibition of Rutabaga root's pulp and peel (Brassica napus L.). Arabian J. Chem. 13, 9, 7078–7086. https://doi.org/10.1016/j.arabjc.2020.07.013.

Stroev, E.A., Makarova, V.G., Matveeva, I.V., 2012. Praktikum po biologicheskoi khimii: Uchebnoe posobie (Practicum in Biological Chemistry: Tutorial). Meditsinskoe Informatsionnoe Agenstvo, Moscow.

Tewari, B.B., 2002. Electrophoretic studies on the chelating tendency of bioactive sulphur-containing amino acids. The metal–methylcysteine–cysteine system. J. Chromatogr. A. 962(1-2), 233–237. https://doi.org/10.1016/s0021-9673(02)00432-6.

Torres-Palazzolo, C.A., Ramírez, D.A., Beretta, V.H., Camargo, A.B., 2021. Matrix effect on phytochemical bioaccessibility. The case of organosulfur compounds in garlic preparations. LWT - Food Sci. Technol. 136,. https://doi.org/10.1016/j.lwt.2020.110301 110301.

Tsai, M.-Ch., Huang, T.-L., 2015. Thiobarbituric acid reactive substances (TBARS) is a state biomarker of oxidative stress in bipolar patients in a manic phase. J. Affect. Disord. 173, 22–26. https://doi.org/10.1016/j.jad.2014.10.045.

Tsopmo, A., 2015. Processing oats and bioactive components. In: Preedy, V. (Ed.), Processing and Impact on Active Components in Food. Academic Press, San Diego, pp. 361–368.

Walters, M.E., Esfandi, R., Tsopmo, A., 2018. Potential of food hydrolyzed proteins and peptides to chelate iron or calcium and enhance their absorption. Foods 7, 172. https://doi.org/10.3390/foods7100172.

Waris, G., Ahsan, H., 2006. Reactive oxygen species: Role in the development of cancer and various chronic conditions. J. Carcinogenesis 5, 14. https://doi.org/10.1186/1477-3163-5-14.

Yehye, W.A., Rahman, N.A., Ariffin, A., Hamid, Sh.B.A., Alhadi, A. A., Kadir, F.A., Yaeghoobi, M., 2015. Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): A review. Eur. J. Med. Chem. 101, 295–312. https://doi.org/10.1016/j.ejmech.2015.06.026.

Ziakas, G.N., Rekka, E.A., Gavalas, A.M., Eleftheriou, P.T., Kourounakis, P.N., 2006. New analogues of butylated hydroxytoluene as anti-inflammatory and antioxidant agents. Bioorg. Med. Chem. 14 (16), 5616–5624. https://doi.org/10.1016/j.bmc.2006.04.030.

Zoghbi, M.G.B., Andrade, E.H.A., Maia, J.G.S., 2002. Volatile constituents from Adenocalymma alliaceum Miers and Petiveria alliacea L., two medicinal herbs of the Amazon. Flavour Fragrance J. 17, 2, 133–135.

Zhu, Y.Z., Huang, S.H., Tan, B.K.H., Sun, J., Whiteman, M., Zhu, Y.-C., 2004. Antioxidants in Chinese herbal medicines: a biochemical perspective. Nat. Prod. Rep. 21 (4), 478–489. https://doi.org/10.1039/b304821g.