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Dedicated to Professor Valer Farcasan
on the occasion of his 95th anniversary

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT ACTIVITY OF A NEW FERROCENYL-IMINE CONTAINING SILOXANE SEQUENCE

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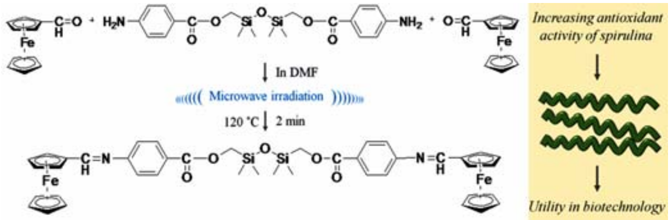
A new ferrocenyl-Schiff base **3** has been obtained by microwave-assisted reaction between formyl-ferrocene **1** and an original siloxane diamine, (1,1,3,3-tetramethyldisiloxane-1,3-diyl)bis(methylene)bis(4-aminobenzoate), **2**. The structure of the resulting Schiff base was confirmed by spectral methods: FT-IR and ^{1}H-NMR. Antioxidant activity of the synthesized compound was evaluated by four different procedures: reduction of phosphomolybdic reagent, on the basis of DPPH$^{\cdot}$ radical, by using the radical cation ABTS$^{+\cdot}$, and reduction of Folin-Ciocalteu reagent. The results evidenced a good activity in biosynthetic processes of *Spirulina platensis* in laboratory conditions.



INTRODUCTION

The area of bioorganometallic chemistry has been rapidly developed in the last time due to the preparation of new organometallic compounds, such as metallocenes, with biological and medical applications.[1,2] Among them, ferrocene has attracted a special attention due to its chemical stability, non-toxicity, interesting redox properties.[3,4] The ferrocenyl-compounds have been evaluated for their anti-inflammatory, antifungal, antiviral or anticancer potential activities.[2] In addition, ferrocene can be easily functionalized for applications in different areas including biology, sensing or catalysis.[5] By attaching to different organic molecules it can provide new properties, can improve catalytic activity and selectivity,[6,7] or may influence the antibacterial and antifungal properties of the resulting compounds.[8-12] Different synthetic strategies have been developed to attach the ferrocenyl moiety to functional groups of various substrates resulting in the internal functions (imine, amide, ester, urea, thiourea or combinations thereof).[13-16] Condensation reaction is a well known procedure that allows to use a large range of functionalized monomers. Our interest was directed towards Schiff bases due to their easily preparation starting from carbonyl and

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amino-functionalized precursors and their applications as ligands in coordination chemistry field,[17,18] in pharmaceutics and medicines.[2]

Different ferrocenyl-Schiff bases and their metal complexes[13,19-21] were prepared: based on salicyloyl and anthraniloyl hydrazide, ferrocenyl-3-(4-methylbenzene)acrylketone, 1-ferrocenyl-3-(4-chlorobenzene)acrylketone, respectively, S-benzyl phenothiazinyl and dithiocarbazate,[21] and have been investigated as antitumoral, anti-bacterial and antiviral agents.[2] The presence of ferrocenyl moiety in the structure of such ligands may affect their donor capacity, which may influence their ability to interact with biomolecules (DNA, aminoacids and peptides).[2] Ferrocenyl-imine compounds containing siloxane sequences: 1,3-bis(3-ferrocenyliminepropyl)tetramethyldisiloxane, α,ω-bis(ferrocenyliminepropyldimethylsiloxy)oligodiphenylsiloxane, and poly[dimethylmethyl(3-ferrocenyliminopropyl)siloxane], have also been reported and investigated in terms of redox and optical properties.[13]

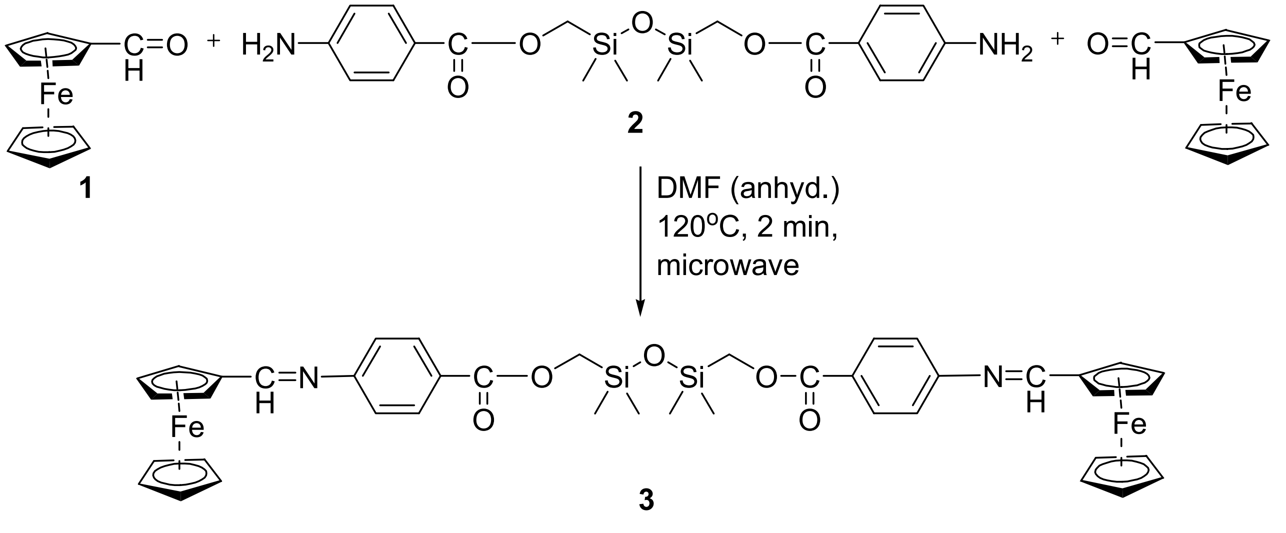
In this work, we describe the synthesis and characterization of a new Schiff base compound containing ferrocenyl and siloxane moieties in the structure by reacting formyl-ferrocene[13] with our new recently published siloxane-containing diamine[22] and the evaluation of this derivative as a promising new antioxidant agent. The microwave-assisted synthesis proved to be the most effective technique for obtaining this compound.

RESULTS AND DISCUSSION

By reacting formyl-ferrocene **1**, and (1,1,3,3-tetramethyldisiloxane-1,3-diyl)bis(methylene)bis(4-aminobenzoate) **2**, in 2:1 molar ratio in DMF, by microwave irradiation, 2 minutes at 120 °C (Scheme 1) resulted the compound **3**, a bis(ferrocenylimine) with asiloxane spacer.

The IR spectrum of the compound **3** shows the presence of at new band at 1627 cm^{-1} due to $\nu_{C=N}$ stretching vibration that emerged due to the condensation of the terminal NH_2 groups of the siloxane diamine **2** and C=O group of the formyl-ferrocene moieties **1**. The peaks at 1697 cm^{-1} ($\nu_{C=O}$ from ester group), 1051 cm^{-1} ($\nu_{Si\text{-}O\text{-}Si}$) and 1254, 839 cm^{-1} ($\nu_{Si\text{-}CH_3}$) are also present in the spectrum of the compound **3** (Fig. 1).

Although the peaks are slightly wider than normal due to the paramagnetic behaviour of the compound **3** containing ferrocenyl units, ^{1}H-NMR spectrum allowed the confirmation of structure. Thus, the signal attributed to the azomethine proton is present at 8.32 ppm. The aromatic protons are shifted from 7.86 ppm and 6.66 ppm in the spectrum of the siloxane diamine[22] to 8.03 ppm and 7.13 ppm in the spectrum of the compound **3**. The signals of ferrocene and of $-CH_2$ protons are present in region 4.80-4.00 ppm, while those of Si-CH_3 protons appear at 0.24 ppm, their intensity ratio being in concordance with the proposed structure (Fig. 2).



Scheme 1 – The reaction path to obtain the compound **3**.

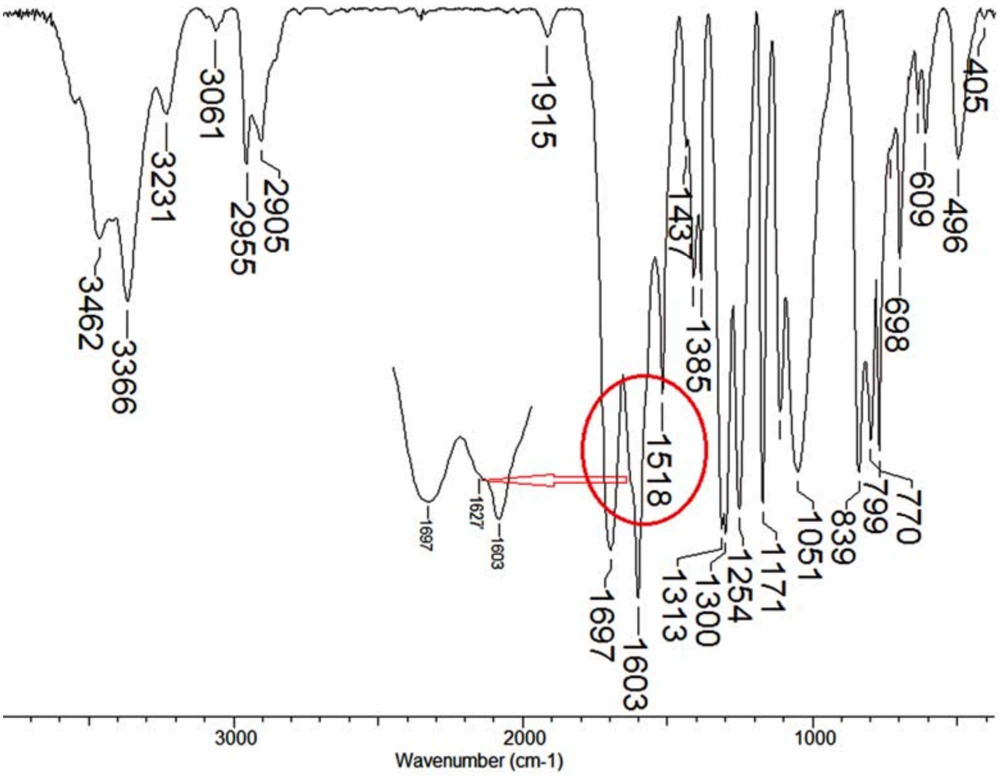


Fig. 1 – FTIR spectrum of the compound **3**.

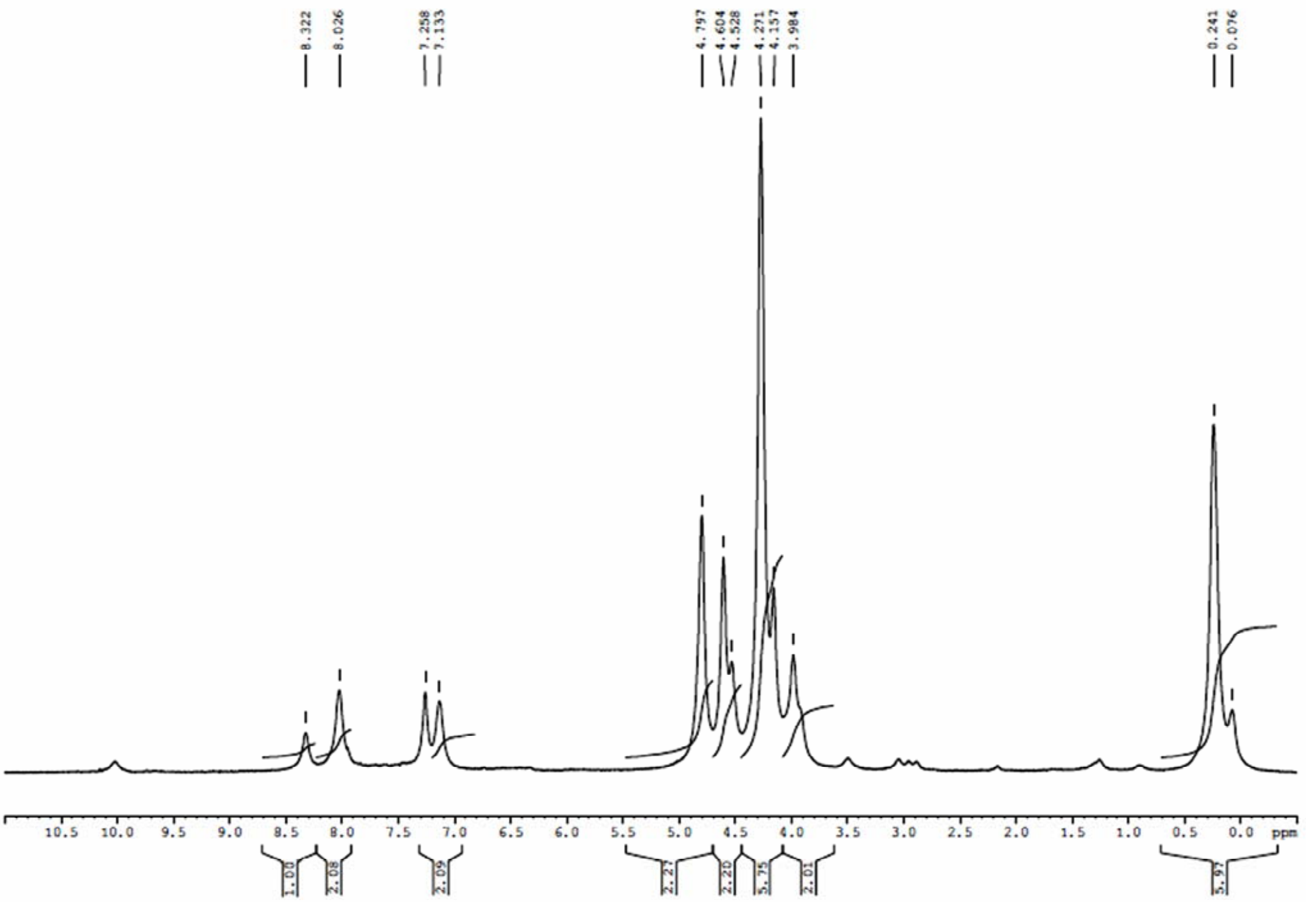


Fig. 2 – ^{1}H-NMR spectrum of the Schiff base **3**.

Antioxidant activity

Recently, more and more research confirms the efficiency of aquatic use in producing raw material for natural antioxidants.[23,24] As sources of antioxidants, cyanobacteria and microalgae such as *Spirulina platensis*, *Chlorella vulgaris, Dunaliella salina, Haematococcus pluvialis* etc. are studied.

Microalgae and cyanobacteria are carriers of antioxidant complex with high activity. Extracts of *Spirulina* are used in medicine to reduce the effects of radiological treatment.[25] *Spirulina platensis* is of particular interest as a source of antioxidants. The biomass of the cyanobacteria contains enzymes (superoxide dismutase, catalase), pigments and vitamins (carotenoids, phycobiliproteins, tocopherol), polysaccaride sulphat and polyene fatty acids.[26] Therefore, *Spirulina* contains antioxidant substances which may form several "lines of defense" against active and less active radicals.[27]

There are many reports in literature regarding the antioxidant activity of different Schiff bases, such as 2-((*o*-hydroxylphenylimino)-methyl)-phenol and 2-((*p*-hydroxylphenylimino)-methyl)-phenol using different sources of water-soluble (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid-Trolox, and L-ascorbic acid) or lipophilic (tocopherol and L-ascorbyl-6-laurate) antioxidants.[28] Ferrocenyl-Shiff bases including *o*-(1-ferrocenylethylideneamino)phenol, *m*-(1-ferrocenylethylideneamino)phenol, *p*-(1-ferrocenylethylideneamino)phenol were also evaluated in 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical (ABTS$^{+\cdot}$). The results obtained in these cases shown that the introduction of ferrocenyl group increased the antioxidant activity more remarkably than benzene-related Schiff bases.[29] Good results were also obtained in the case of ferrocene carbohydrate conjugates.[30]

In order to study the influence of the compound **3** on the antioxidant potential of *Spirulina platensis* biomass, four methods were applied: evaluation of antioxidant capacity by reduction of phosphomolybdic reagent[31], determination of antiradical capacity by using DPPH$^{\cdot}$ radical,[32] determination of the antiradical capacity by using of radical cation ABTS$^{+\cdot}$,[33] and evaluation of phenols content using the method of reduction of Folin-Ciocalteu reagent.[34] Two concentrations, of 0.1 mg/L and 1.0 mg/L, of the compound **3** were used. Comparative results of the tests were expressed as % of control sample and are summarized in Table 1. The control sample is *Spirulina* culture cultivated under identical conditions in the absence of the supplement environment of the culture.

To evaluate the change of antioxidant activity of cyanobacterial biomass, antioxidants tests have been applied for two types (water and ethanol (96%)) of extracts. The compound **3** has the ability to change the antioxidant character of the biomass. The antioxidant test with phosphomolybdic reagent as substrate can be used as test for determination of the total antioxidant activity. In the obtained water extracts from *Spirulina* biomass at a concentration of 0.1 mg/L, the compound **3** determined an increase of the antioxidant activity with 42%.

Tests with application of DPPH$^{\cdot}$ and ABTS$^{+\cdot}$ radicals caused an increase in antioxidant activity of the water extracts with 40% at a concentration of 0.1 mg/L of the compound **3** and with 44% at a concentration of 1.0 mg/L. Antioxidant activity (ABTS test) of ethanol extract increase with 32% at a concentration of 1.0 mg/L.

Table 1

Modification of antioxidant activity (%) of the extracts (water and ethanolic 96%) from *Spirulina* biomass obtained by supplementation of the mineral medium with the compound **3**

Extract	**Phosphomolybdate Test**[(a)]		**DPPH·**[(b)]		**ABTS$^{+\cdot}$**[(c)]		**Folin-Ciocalteu Test**[(d)]	
	Concentration of the compound **3** in mineral medium, mg/L							
	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.
Water	42%	n.m. [(e)]	40%	n.m	n.m.	44%	30%	100%
Ethanol 96%	17%	n.m.	n.m.	n.m	32%	n.m	n.m.	n.m.

[(a)]evaluation of antioxidant capacity by reduction of phosphomolybdic reagent, [(b)]determination of antioxidant capacity by using DPPH· radical[1], [(c)]determination of the antioxidant capacity by using of radical cation ABTS$^{+\cdot}$, [(d)]evaluation of phenols content using the method of reduction of Folin-Ciocalteu reagent, [(e)]non modification.

The accumulation of phenols is an important property for the biomass of *Spirulina* and increases by stimulation of the biosynthetic activity. The compound applied to the cultivation of *Spirulina* biomass induced synthesis of phenols. The concentration of 0.1 mg/L compound **3** caused a 30% increase in the content of phenols in water extract. The concentration of 1.0 mg/L doubled the content of phenols in *Spirulina* biomass.

EXPERIMENTAL

Materials

Formyl-ferrocene (**1**) was prepared according to the method described in literature.[13] The siloxane diamine, (1,1,3,3-tetramethyldisiloxane-1,3-diyl)bis(methylene)bis(4-aminobenzoate), (**2**), was prepared according to the procedure described in ref.[22] The solvents: anhydrous dimethylformamide (Aldrich), chloroform (Chimopar S.A.), ethanol (Chimopar S.A.) were used as received.

Spirulina platensis strain CNMN-CB-11 (Cyanophyta), stored in National Collection of Nonpathogenic Microarganisms, Institute of Microbiology and Biotechnology, Academy of Sciences of Moldova, was grown on mineral nutrient medium with the following chemical composition:

- Macroelements (g/L): $NaNO_3$-2.25; $NaHCO_3$-8.0; NaCl-1.01; K_2SO_4-0.3; $NaHPO_4$-0.20; $MgSO_4\cdot 7H_2O$-0.20; $CaCl_2$-0.024.

- Microelements solution (1 ml/L), containing (mg/L): H_3BO_3-2.86; $MnCl_2\cdot 4H_2O$ -1.81; $ZnSO_4\cdot 7H_2O$- 0.22; $CuSO_4\cdot 5H_2O$- 0.08; MoO_3-0.015; Fe-EDTA, 1.0 ml/L.

The cultivation was carried out at pH 8-9, temperature of 30-32 °C and illumination of 3000-4000 lx. The amount of inoculum was 0.40-0.45 g/L. The culture was stirred frequently. The duration of cultivation was 144 hours. From the biomass collected during the experiences results, water and ethanol (96%) extracts were prepared. For obtaining the extracts, one gram of biomass native (*Spirulina platensis*) mixed with 100 mL of water or ethanol for 2 hours. The extracts were isolated from the biomass and stored at 0 °C.

Techniques

The microwave experiment in seal vessel was carried out in a PC operated (Synergy software) Discover LabMate – CEM, Inc. monomodal reactor equipped with the IntellyVent pressure controller and magnetic stirring ability.

Elemental (C, H, N) analyses were obtained with a Perkin–Elmer CHNS 2400 II elemental analyser.

Infrared (IR) absorption spectra, as KBr pellets of the prepared compounds, were recorded on a Bruker Vertex 70 FT-IR spectrometer. Registrations were performed in the range of 400–4000 cm^{-1}, in the transmission mode, at room temperature with a resolution of 2 cm^{-1} and accumulation of 32 scans.

The NMR spectra were recorded on a Bruker Avance DRX 400 MHz Spectrometer (Bruker, Rheinstetten, Germany) equipped with a 5mm QNP direct detection probe and z-gradients. Spectra were recorded in $CDCl_3$ at room temperature. The chemical shifts are reported as δ values (ppm).

UV-Vis spectrophotometer (PG Instruments Limited, Alma Park, Wibtoft, England) was used to estimate the extinction of the samples in antioxidant activity tests. Determination of productivity for *Spirulina platensis* was photometric performed by recalculating the cell mass to the absolute dry biomass.[35]

Procedure

The classical thermal synthesis

The compound **3** was prepared first by reacting formyl-ferrocene (0.214 g, 1 mmol), with (1,1,3,3-tetramethyldisiloxane-1,3-diyl)bis(methylene)bis(4-aminobenzoate) (0.216g, 0.5 mmol) in absolute ethanol (20 mL) at reflux for 4 hours under argon atmosphere. The product resulted after the evaporation of the solvent was analysed by FT-IR and ^{1}H-NMR techniques indicating unreacted diamine and formyl-ferrocene.

The microwave-assisted synthesis

Formyl-ferrocene (0.107 g, 0.5 mmol) in anhydrous DMF (4 mL) was added to a solution of (1,1,3,3-tetramethyldisiloxane-1,3-diyl)bis(methylene)bis(4-aminobenzoate) (0.108 g, 0.25 mmol) in anhydrous DMF (4 mL) in a 10 mL microwave vial equipped with magnetic stirrer. The reaction vial was sealed and placed in the microwave reactor and heated to 120 °C for 2 minutes. After cooling to room temperature, the product was poured in distilled water (30 mL) and extracted with chloroform (3x10 mL). The organic layer was vacuum distilled, resulting a brown powder (0.215 g, 52% yield). Anal. Calcd for $C_{46}H_{56}N_2O_5Si_2Fe_2$ (M_r 884.24 g/mol): C, 62.44; H, 6.38; N, 3.17. Found: C, 62.24; H, 6.37; N, 3.29.

IR ν_{max} (KBr): 3462m, 3366m, 3231w, 2955w, 2905w, 1697vs, 1627s, 1603vs, 1518s, 1437w, 1410m, 1385m, 1313s, 1300s, 1254s, 1171s, 1111s, 1051s, 839s, 799s, 770s, 729w, 698m, 636w, 609w, 496w.

^{1}H-RMN ($CDCl_3$, 400 MHz, ppm: 8.32 (s, 2H, -C*H*=N-), 8.03 (s, 4H, Ar-*H*), 7.13 (s, 4H, Ar-*H*), 4.80, 4.60 (8H, $-C_5H_4$), 4.27 (10 H,$-C_5H_5$), 4.00 (s, 4H, $-Si-CH_2-$), 0.24 (s, 12H, $-Si-CH_3$).

Antioxidant activity evaluation of the *Spirulina* water and ethanol extracts

Evaluation of antioxidant activity by reducing phosphomolybdic reagent

The antioxidant activity was determined indirectly, spectrophotometric, in the redox system consisting in the reduction of Mo(VI) to Mo(V) with the formation of phosphate green/Mo(V), the electrons transfer occurring in acid environment.[31]

Determination of antioxidant activity by using DPPH radical

The DPPH$^{\cdot}$ (1,1-diphenyl-2-picrylhydrazyl) radical, used as a substrate was reduced by addition of hydrogen atoms to give 1,1-diphenyl-2-picrylhydrazine, color changing from purple to yellow. The concentration of DPPH$^{\cdot}$ radical in the working solution and the reaction duration are individual and are determined for each case depending on the nature of the antioxidant and the solvent used.[32]

Determination of the antioxidant activity by using the radical cation ABTS$^{+\cdot}$

The method to test the antiradical capacity using ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6 sulfonic acid)] is well known to determine the antioxidant activity of different samples.[33] ABTS radical was generated by the oxidation of ABTS with potassium persulphate.

Evaluation of phenols content using the method of reduction of Folin-Ciocalteu reagent

The method is based on the electron transfer that occurs in alkaline medium with the reduction of the phosphomolybdic/phosphowolframic acid complex which is colorimetrically determined.[34]

CONCLUSIONS

The microwave-assisted condensation of the formyl-ferrocene with (1,1,3,3-tetramethyldisiloxane-1,3-diyl)bis(methylene)bis(4-aminobenzoate) led to the formation of corresponding Schiff base, as analyses by FTIR and ^{1}H-NMR techniques indicated. The study of the antioxidant activity of the product was carried out by four methods. Analysis of the results showed the ability of this compound to change the antioxidant status of *Spirulina*. The antioxidant capacity values thus determined recommend the application of this compound in the development of new technologies for obtaining antioxidant complexes soluble in water and ethanol. The compound changes both from quantitative and qualitative point of view the antioxidant components from Spirulina biomass, is actively involved in biosynthetic processes of the cyanobacteria stimulating the synthesis of phenols in biomass.

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