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*V.A. Litvin^a, R.A. Njoh^b***QUERCETIN AS A PRECURSOR IN THE SYNTHESIS OF ANALOGUES OF FULVIC ACIDS AND THEIR ANTIBACTERIAL PROPERTIES**^a **The Bohdan Khmelnytsky National University of Cherkasy, Cherkasy, Ukraine**^b **Institute of Health Sciences, Department of Toxicology, Near East University, Nicosia, Cyprus**

A simple, fast and effective method for producing synthetic substances with properties similar to natural humic substances has been proposed. The synthesis method is based on the oxidation of quercetin by molecular oxygen in an alkaline medium, followed by conversion to the acid form by passing through a cation exchange column. Study of elemental and functional compositions, spectral properties (UV/Vis and IR range) and redox characteristics allowed qualifying the resulting product as a synthetic fulvic acid. The enhanced antibacterial properties of the obtained synthetic product were established. The minimum concentration of inhibition of synthetic fulvic acid derived from quercetin is 25 $\mu\text{g mL}^{-1}$, which is in 100 times less than for natural humic substances.

Keywords: quercetin, precursor, synthetic humic substance, fulvic acid, antibacterial properties.

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Introduction

Humic substances (HSs) are a combination of biothermodynamically stable compounds formed during the decomposition and transformation of plant and animal residues. HSs have no analogues in living organisms and are distinguished by dark color, polydispersity and high molecular weights [1]. The stochastic nature of HSs is their specificity, which is caused by the characteristic features of their formation as a result of the natural selection of stable structures. Thus, the fundamental properties of HSs include non-stoichiometric composition, irregular structure, heterogeneity of structural elements and polydispersity [2].

The interest in these substances is due to a wide range of therapeutic effects, as confirmed by experimental studies of recent years [3,4]. Specific organic substances have adaptogenic, antitoxic, antimicrobial, diuretic, anti-inflammatory effects; they are a universal matrix that allows the necessary trace elements to enter into the human body in an active form. However, the main difficulty in creating therapeutic humic drugs is the lack of production control methods and quality control of the resulting product, since the structure, fractional composition and, accordingly, the properties of natural humic substances strongly depend on the method of

isolation, modification and source of their production [2]. To solve this problem, synthetic analogues of natural HSs can be used, the synthesis of which is carried out under strictly controlled conditions, so that they are characterized by reproducible and standardized properties. In addition, it is possible to prepare a product with certain therapeutic properties by changing the nature of the low molecular weight precursor.

Taking into account the chemical structure of natural HSs, the main methods for producing synthetic analogues are based on the oxidation reactions of different phenolic compounds. In particular, known methods for producing HSs are oxidation reactions between catechol and acetic acid [5], catechol and amino-acid [6], glucose, glycine and catechol [7], gallic acid [8], 3,4-dihydroxy-toluene, 3,4-dihydroxyphenylacetic acid, caffeic acid, hydrocaffeic acid, chlorogenic acid, adrenaline acid, adrenaline gentisic acid [9], and hematoxylin [10]. Pure oxygen, air, Ag_2O , NaIO_4 , H_2O_2 , $\text{K}_2\text{S}_2\text{O}_8$, and enzymes are used as oxidizing agents [5–10].

The main disadvantages of the known methods for producing synthetic humic substances are as follows: (i) the use of toxic substances as oxidizing agents, which limits the possibility of using the final product; (ii) multi-stage process, which requires a

long synthesis time (sometimes up to 1 month); and (iii) incomplete compliance of the properties of the obtained product with natural organic substances.

Therefore, research on the development of new, technologically simple and effective methods for producing synthetic humic substances and drugs based on them is an urgent and promising task.

In this study, a new original method for producing synthetic fulvic acid from quercetin (SFAQ) is presented. The method is characterized by simplicity of synthesis, speed, lack of toxic by-products and reproducibility of the main characteristics. We conducted a comprehensive study of the physicochemical and antibacterial properties of synthetic fulvic acids derived from quercetin.

Experimental

Chemicals

Quercetin (99% purity) was purchased from Sigma Aldrich (Germany). Other chemicals were of analytical grade (Merck, Germany). Deionized water from Milli-Q system (Millipore) was used for all sample preparation and dilutions.

Synthesis of the synthetic fulvic acid from quercetin

To prepare synthetic analogs of fulvic acid, 1 g of quercetin was placed in 10 mL plastic container and introduced into a 2 L reaction flask. After that, 13 mL of 1 M NaOH solution and 70 mL of distilled water were carefully added. After sealing the system, air from the reaction flask was displaced with pure oxygen and automatic reagent mixing was turned on. At a temperature of 25°C and normal atmospheric pressure, the process of oxidative polymerization was completed in 90 minutes. The resulting solution was mixed with cation exchange resin in the H-form, and then filtered. To remove carbonate acid, a solution of synthetic fulvic acids (SFAQ) was heated to 80°C for 15 minutes. The solution of SFAQ was dried at 80°C.

Elemental analysis

Dried preparations of SFAQ were analyzed for C and H using Carlo Erba 1106 CHN-analyzer. The content of oxygen was calculated by the difference: $O(\%) = 100\% - (C(\%) + H(\%))$. The H:C and O:C atomic ratios were also calculated.

UV-Visible spectra

UV-VIS absorption spectra were registered by Lambda 35 Perkin Elmer UV-visible spectrophotometer in the range of 200–600 nm. Quartz cuvettes (1 cm wide) were used.

FTIR spectra

The FTIR spectra of SFAQ from quercetin were recorded by the Perkin-Elmer spectrometer (SpectrumGX) with a resolution of 2 cm⁻¹ over a scan range of 4000–500 cm⁻¹ using KBr pellets

(3–6 mg FA per 5 mg KBr).

XRD analyses

X-ray powder diffraction patterns of synthetic fulvic acid were obtained using FeK_α radiation (the wavelength of 0.19360 nm) on a DRON-2 X-ray diffractometer equipped (LOMO, Russia). The diffraction pattern was scanned over $2\theta = 20^{\circ} - 120^{\circ}$ at a scanning rate of 0.01° s⁻¹.

Scanning electron microscope (SEM) analysis

Scanning electron microscope (SEM-100) was used to study the surface morphology of SFAQ. For measurements, the powder of SFAQ was applied to a conductive tape and placed in a microscope chamber.

Acidic functional groups

Total acidity was determined by the baryta adsorption method and carboxyl groups were determined by the Ca-acetate method. The OH-groups content was calculated as the difference between total acidity and COOH-groups content.

Reducing capacity

Reducing capacity of SFAQ was determined using ferricyanide as an oxidizing agent. To this end, the absorption spectra of SFAQ solution with concentration of 50 mg L⁻¹, a solution of K₃Fe(CN)₆ with concentration of 0.25 mmol L⁻¹ and a solution containing both SFAQ and K₃Fe(CN)₆ in the same concentrations were recorded using a Lambda 35 Perkin Elmer UV-visible spectrophotometer. All solutions were prepared in buffer with pH 7. The resulting solutions were kept in the dark for 24 hours at room temperature. The absorbance of each solution at 420 nm was used to calculate the reducing capacities as follows:

$$\text{reducing capacity (mmol g}^{-1}\text{)} = \frac{\Delta C(\text{K}_3\text{Fe}(\text{CN})_6)}{C_{\text{FA}}}$$

where $\Delta C(\text{K}_3\text{Fe}(\text{CN})_6)$ is the decrease in concentration of K₃Fe(CN)₆ (mmol L⁻¹); and C_{FA} is the mass concentration of fulvic acid in the control solution (g L⁻¹).

EPR analysis

EPR spectra of solid synthetic fulvic acid were measured using a 2 mm quartz tube. To calculate the spin content in the sample, a spin standard CuCl₂·H₂O was used. EPR experiments were carried out using a Bruker ESP 300 E spectrometer operating in the X-band and equipped with 100 kHz field modulation.

Antimicrobial test

The antibacterial activities of SFAQ were determined by disc diffusion method against pathogenic bacteria *Escherichia coli*, *Pseudomonas*

aeruginosa (gram-negative), and *Staphylococcus aureus* (gram-positive). Soya bean casein digest agar plates were prepared, sterilized and solidified. After solidification, bacterial cultures were swabbed on these plates. The sterile disc was dipped in fulvic acid solution ($100 \mu\text{g mL}^{-1}$) and placed in the agar plate and kept for incubation at 37°C for 24 hours. The zone of inhibition of bacteria was measured. The experiments were repeated thrice and the mean values of zone diameter were presented.

Minimum inhibitory concentration (MIC)

To determine the MIC of SFAQ, the serial dilution method in a liquid nutrient medium (broth) was used. A stock solution was prepared with a specific concentration of synthetic fulvic acids. A series of decreasing double dilutions of SFAQ was prepared from it in the wells of a broth plate Mueller Hinton and the test culture was added (10^5 – 10^6 bacterial cells). The control was a well with a broth and culture without SFAQ. Incubation was carried out during 24 hours. MIC was determined, which corresponded to the concentration of the drug in the last well with visible growth retardation (transparent nutrient medium).

Results and discussion

Synthesis of the synthetic fulvic acid from quercetin

Quercetin is one of the most common compounds of plant origin with a variety of medicinal properties and high antioxidant activity. It is found in many fruits, vegetables, leaves and grains [11]. The presence of a large number of phenolic groups in the structure of quercetin makes it a good candidate for the use as a precursor in the preparation of synthetic humic substances. The distribution of electric charge in the quercetin molecule (Fig. 1)

indicates rather strong negative charge at the oxygen atom of hydroxyl groups, which agrees with common features of the typical fulvic acid components.

Synthetic fulvic acids (SFAQ) were obtained in oxidation reaction of quercetin with molecular oxygen at pH 12. The use of pure oxygen and strongly alkaline medium accelerates the synthesis process. The oxidation process was controlled by monitoring the amount of oxygen consumed in the reaction. The cessation of oxygen consumption indicated the completion of the synthesis process. Under these conditions, the process lasted for 80 minutes. Since the reaction was carried out in a strongly alkaline medium, the sodium salt of fulvic acid was ultimately formed. In order to convert it into the acid form, cation exchange resin in the H^+ -form was used. The resulting solution of synthetic fulvic acids has pH 2.3. Since the obtained product dissolves at any value of pH, this indicates that the synthetic fulvic acid was obtained, and not humic acid, which precipitates in an acidic medium. The synthetic fulvic acid derivate derived from quercetin (SFAQ) was also soluble in water, alkali, methanol, ethanol and dimethyl sulfoxide.

A comprehensive study of the physicochemical properties of the resulting product was carried out in order to confirm their similarity with natural fulvic acids.

Elemental analysis

The elemental composition of humic substances is one of the important indicators by which their structure and properties can be well understood. Differences in the elemental composition of natural humic substances are due to the nature and structure of humic acids as well as to the dependence on the

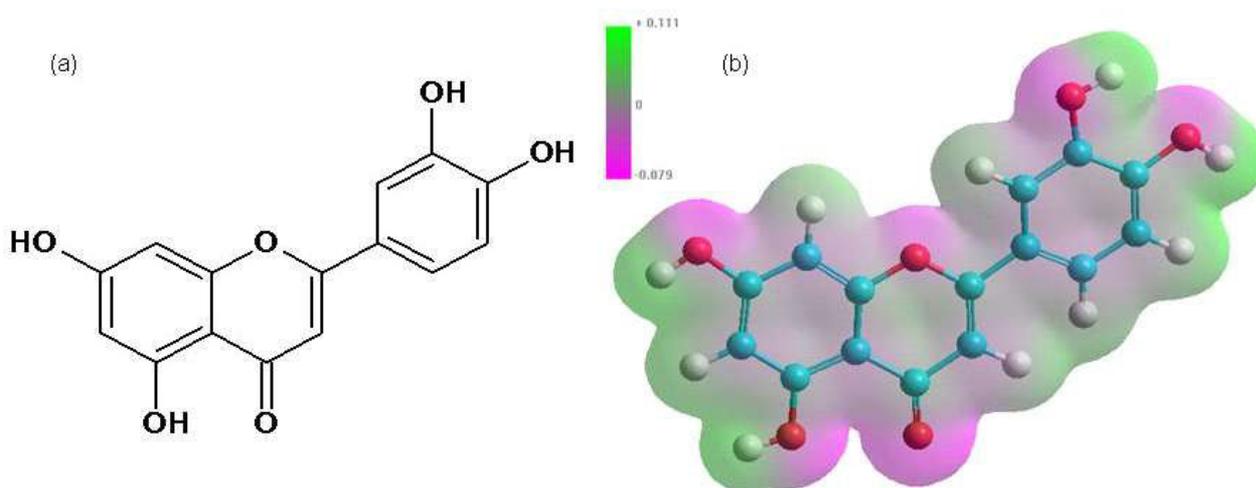


Fig. 1. (a) – Chemical structure of quercetin; and (b) – distribution of electrostatic potential calculated with semiempirical PM3 method

source [2,12].

The study of the elemental composition of SFAQ showed that the percentage of carbon, oxygen and hydrogen is in the range, which is typical of natural humic substances [2]. The corresponding data on the elemental composition of quercetin and SFAQ, expressed in mass percent, are presented in Table 1.

Orlov showed [13] that the expression of the elemental composition of humic substances in mass percent does not give a correct and complete idea of the role of individual elements in the structure of matter. Indicators that are more accurate can be obtained using the atomic ratio H/C and O/C, the analysis of which allows judging the ratio of aromatic and aliphatic structures in the SFAQ molecules. An increase in H/C from 0.67 to 1.09 during the transition from quercetin to SFAQ indicates the appearance of aliphatic fragments in the structure. At the same time, an increase in the O/C ratio indicates an increase in the number of oxygen-containing groups. The evident decrease in the percentage of C indicates a decarboxylation process.

Spectral characterization of the synthetic fulvic acid derived from quercetin

Absorption spectra

UV-Vis spectroscopy is a valuable method for identifying chromophore functional groups of individual organic compounds. In the case of complex multicomponent objects, which include HSs, the possibilities of this method are limited, because their spectra are the result of overlapping maxima of various chromophores and do not allow obtaining information on the functional group composition.

The absorption spectrum of SFAQ (Fig. 2) is typical of natural fulvic acids. In the range from 200 to 600 nm, the spectrum is a gentle descending curve without any extremums. The absorption maxima at 260 and 370 nm in the absorption spectrum of quercetin disappear in the absorption spectrum of SFAQ.

To characterize the HSs, it is customary to use the magnitude of the extinction coefficient and the ratio of optical densities at 465 and 650 nm, the so-called color coefficient (E_{465}/E_{650}). The values $E_{465}^{0.001\%}$

and E_{465}/E_{650} for SFAQ are 0.02 and 4.88, respectively, which are in the range typical of fulvic acids [13].

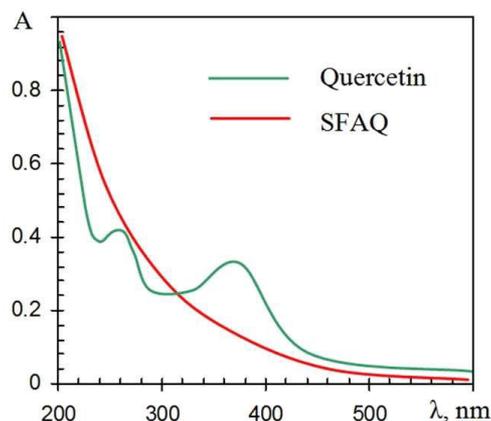


Fig. 2. The UV/Vis spectra of quercetin and synthetic fulvic acid derived from quercetin

FTIR spectroscopy

The use of IR spectroscopy for the analysis of HSs is complicated by the intense overlap of the absorption bands of the functional groups due to the heterogeneity of their chemical environment. Therefore, the spectral information that can be obtained from the IR spectra of HSs is limited by the identification of the main functional groups [12].

FTIR spectra were used as a tool to study the changes in absorption bands and therefore in functional groups in SFAQ in comparison with the starting material (quercetin) and natural humic substances (Fig. 3).

The obtained FTIR spectrum of SFAQ shows a close similarity to that of natural HSs. The main absorption maxima, which are characteristic of natural HSs, are found in the spectrum of the synthetic analog of fulvic acid obtained from quercetin. This indicates the proximity of their chemical structure.

The peaks on FTIR spectrum of quercetin at 3356 cm^{-1} correspond to the stretching vibration of OH groups involved in hydrogen bonding. The absorption at 1654 cm^{-1} is attributed to C=O groups. Bands at 1610 cm^{-1} , 1560 cm^{-1} and 1510 cm^{-1} are

Table 1

Elemental composition (%) and atomic ratios H/C and O/C of quercetin, synthetic fulvic acids (SFAQ) and natural humic substances (HSs)

Substances	C, %	O, %	H, %	H/C	O/C
Quercetin	59.60	37.09	3.31	0.67	0.47
SFAQ	46.44	49.35	4.21	1.09	0.80
Natural HSs [12]	35.1–75.4	31.1–54.5	3.2–7.0	0.85–1.84	0.38–1.07

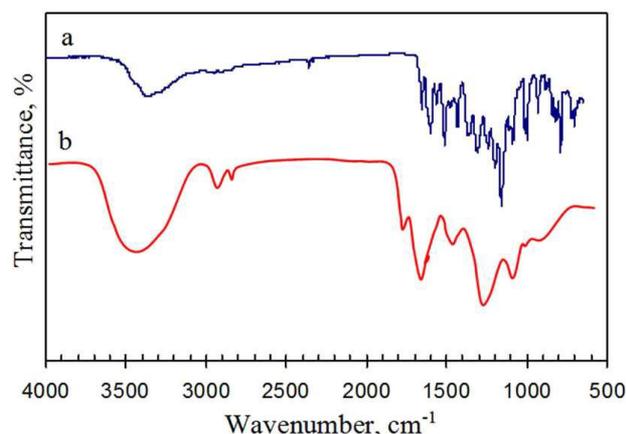


Fig. 3. FTIR spectra of quercetin (a) and synthetic fulvic acid derived from quercetin (b)

associated with the aromatic ring C=C. The peak at 1317 cm^{-1} belong to the in-plane bending band of C-H in aromatic hydrocarbon; out-of-plane bending bands are evident at 933 cm^{-1} , 820 cm^{-1} , 679 cm^{-1} and 600 cm^{-1} . The maxima at 1263 cm^{-1} , 1200 cm^{-1} and 1165 cm^{-1} correspond to the C-O stretching in the aryl ether ring, the C-O stretching in phenol and the C-CO-C stretch and bending in ketone, respectively.

The FTIR spectrum of the obtained synthetic fulvic acids is shown in Fig. 3. It can be seen that after oxidative condensation most of the bands are broadened and shifted as compared with those of pure quercetin. The spectrum shows some characteristic and strong absorption bands in the regions of $3400\text{--}3200\text{ cm}^{-1}$, $1850\text{--}2920\text{ cm}^{-1}$, 1720 cm^{-1} , $1600\text{--}1640\text{ cm}^{-1}$, $1220\text{--}1260\text{ cm}^{-1}$ and $900\text{--}1070\text{ cm}^{-1}$. A wide absorption band at 3450 cm^{-1} exhibits the presence of OH groups bound by inter molecular hydrogen bonds. The bands at 2920 cm^{-1} and 2850 cm^{-1} correspond to the stretching vibrations of CH_2 and CH_3 groups in the aliphatic chain, and absorption at 1374 cm^{-1} is ascribed to the deformation vibrations of CH in a saturated aliphatic radical. The absorption band of medium intensity at 1718 cm^{-1} shows the presence of -C=O in the carboxyl and partially in the carbonyl groups. A peak at 1645 cm^{-1} is characteristic of the stretching vibrations of the -C=C- bond conjugated with the C=O or COOH groups, and the presence of a band at 1600 cm^{-1} corresponds to the -C=C- bond of the aromatic ring forming the central (frame) part of fulvic acids. The plane deformation vibrations of CH ($1070\text{--}900\text{ cm}^{-1}$) indicate various types of substitution of the benzene ring. The band at 1228 cm^{-1} refers to the stretching vibrations of CO of phenolic and carboxyl groups, and strong absorption at 1124 cm^{-1}

corresponds to the stretching vibrations of CO bond of primary and secondary alcohol groups. These bands are due to the presence of various oxygen-containing functional groups in the structure of SFAQ.

X-ray diffraction pattern

The absence of diffraction peaks typical of quercetin in the X-ray pattern of the SFAQ demonstrates that quercetin was completely converted into synthetic fulvic acids during the synthesis (Fig. 4). This also confirms that synthetic fulvic acids are mainly constituted by a mixture of amorphous solid compounds. The amorphous nature is also characteristic of natural fulvic acids [13], which is an additional confirmation of the similarity of their properties.

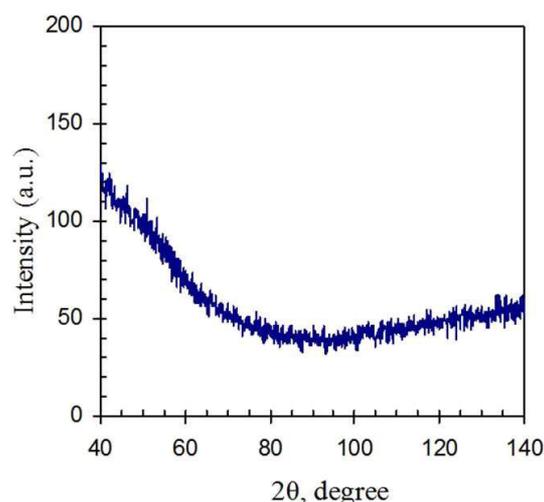


Fig. 4. X-ray powder diffraction patterns of synthetic fulvic acid derived from quercetin

SEM analysis

The scanning electron micrographs of SFAQ showed a loose spongy structure of humic acids with the particles tending to aggregate to each other (Fig. 5).

Acidity of synthetic fulvic acid derived from quercetin

It is known that there are more than ten different types of oxygen, nitrogen and sulfur-containing functional groups in natural HSs: carboxyl, phenolic and alcohol hydroxyl, carbonyl, quinone, methoxy, ester, enol, amino, amido and imido groups, thiol and disulfide groups. However, the chemical behavior of fulvic acids is determined mainly by oxygen-containing functional groups.

Fulvic and humic acids form poorly soluble salts with alkaline earth metals. This property is widely

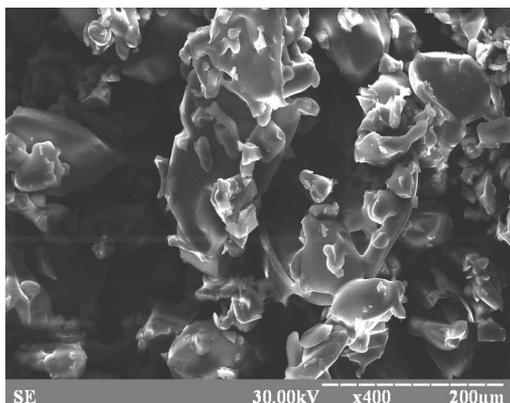
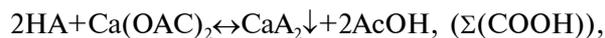
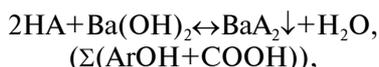


Fig. 5. SEM image of synthetic fulvic acid derived from quercetin

used in traditional methods for the determination of acid groups in samples by the reactions with barium hydroxide and calcium acetate:



where A stands for the acid residue of fulvic acids.

The difference between the results of two testing gives $\Sigma(\text{ArOH})$.

To determine the number of reacted groups after precipitation of fulvates in the barite method, the excess of $\text{Ba}(\text{OH})_2$ is titrated with HCl solution. In the Ca-acetate method, the amount of acetic acid released as a result of the reaction is determined. Despite the formation of slightly soluble compounds, both reactions are highly reversible and a substantial excess of the reagent is required to achieve complete conversion [12]. In addition, the removal of fulvic acid from the solution in the form of a precipitate of barium or calcium fulvates leads to a shift of the equilibrium to the right and, therefore, to a more complete determination of the groups than by potentiometric titration.

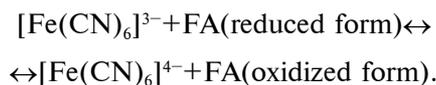
The content of functional groups in natural HSs varies depending on the source of origin [12]. In particular, humic substances extracted from water are usually characterized by a high content of carboxyl

groups [12]. However, the following tendency is more clearly pronounced: fulvic acids exhibit a higher content of carboxyl groups than humic acid, regardless of the source of origin.

The obtained data on the content of carboxyl and phenolic groups in the synthetic fulvic acid (Table 2) are in the range that is characteristic of natural humic substances. However, the advantage of the resulting synthetic product is the reproducible amount of acid groups.

Redox properties of synthetic fulvic acid derived from quercetin

The functional groups responsible for electron transfer reactions involving HSs are quinoid fragments, which form free radicals (semiquinones) upon single-electron reduction [14]. To determine the reduction capacity of synthetic fulvic acids, ferricyanide ($[\text{Fe}(\text{CN})_6]^{3-}$) was used as an oxidizing agent. The reduction of ferricyanide by synthetic fulvic acid can be represented by the following equation:



The use of ferricyanide is associated with the fact that it has a constant redox potential (~ 430 mV) in a wide pH range from 4 to 11 and a high complex stability ($K=9.1 \cdot 10^{41}$). Ferricyanide reacts via one-electron reduction yielding $[\text{Fe}(\text{CN})_6]^{4-}$, which can be followed by UV-Vis spectroscopy (the absorption maximum of $[\text{Fe}(\text{CN})_6]^{3-}$ is observed at 420 nm).

The reducing capacity of synthetic fulvic acid from quercetin is 3.07 ± 0.2 mmol g^{-1} . The obtained value is in the range which is characteristic of natural humic substances (1.09 – 6.5 mmol g^{-1}) as well as of partially oxidized natural humic substances and copolymers of humic substances with hydroquinone and catechol (1 – 4 mmol g^{-1}) [14].

EPR spectral analysis

The EPR spectrum of SFAQ (Fig. 6) demonstrates one intense broad line at $g=2.0033$, which indicates a clear presence of free radicals in the structure of SFAQ. The concentration of paramagnetic centers is $4.8 \cdot 10^{16}$ spin g^{-1} and the line half-width is 3.6 Gauss. These data agree with

Table 2

The functional analyses of SFAQ in comparison with natural HSs

Humic substances	$\Sigma(\text{ArOH} + \text{COOH}), \text{mmol g}^{-1}$	$-\text{COOH}, \text{mmol g}^{-1}$	$\text{ArOH}, \text{mmol g}^{-1}$
SFAQ	$7.8 \pm 0.1 / 2.8^*$	$5.0 \pm 0.2 / 2.8^*$	$2.8 \pm 0.1 / 3.0^*$
Natural HSs [13]	4.7–10.3	2.6–8.2	1.6–3.9

* Note: variation factor.

previously reported g-values for humic substances and are consistent with semiquinones being the primary organic radicals.

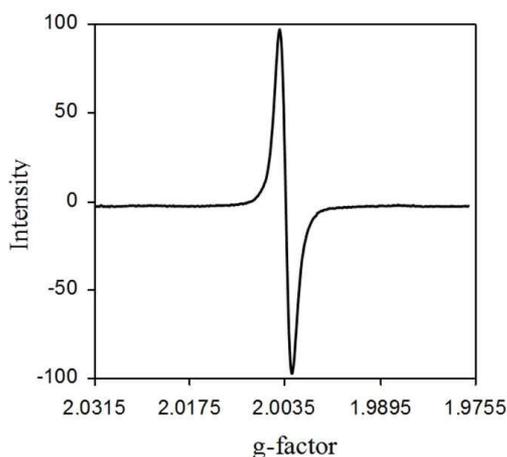


Fig. 6. EPR spectrum of synthetic fulvic acid derived from quercetin

The determination of the number of paramagnetic centers makes it possible to calculate the conditional average statistical molecular weight, provided that a simple EPR waveform implies that there is only one unpaired electron in the molecule. The calculation of molecular weight by the content of paramagnetic centers has a definite advantage over other methods, since it characterizes the solid state of fulvic acids, while the molecular weight determined by other methods refers to macro-localities in solution. The consequence of this may be a reduced molecular weight of fulvic acids as compared with the values characterizing their solid state.

Molecular weight was calculated by the formula:

$$M = N_0 / N_e,$$

where M is the molecular weight, N_0 is the Avogadro number ($6.02 \cdot 10^{23}$), and N_e is the number of paramagnetic centers per 1 g of the substance.

The calculated molar weight of SFAQ is $1.25 \cdot 10^7$.

Antimicrobial activity analysis

The antibacterial effect of the SFAQ was evaluated by disc diffusion method. The diameter of inhibition zones (in millimeters) around the different disks against test strains is shown in Table 3. The results suggest that the SFAQ acids inhibit both gram-positive and gram-negative bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*).

Table 3

Antimicrobial activity of synthetic humic substances (mean \pm SD, n=3)

Microorganism	Diameter of the inhibition zone (mm)
<i>Escherichia coli</i>	12.1 \pm 0.2
<i>Pseudomonas aeruginosa</i>	15.5 \pm 0.2
<i>Staphylococcus aureus</i>	13.8 \pm 0.1

The minimum concentration of inhibition of SFAQ derived from quercetin is $25 \mu\text{g mL}^{-1}$, which is 100 times less than for natural humic substances and 1.5 times less than for model humus substances obtained synthetically by oxidation of hydroquinone and pyrocatechin [15].

The efficiency of synthetic humic substances against pathogenic microorganisms in vitro provides a rational basis for the therapeutic use of synthetic analogues of fulvic acids in medicine.

Conclusions

We developed a novel approach to preparation of synthetic fulvic acid from quercetin using molecular oxygen in an alkaline solution. Comparative analysis of the elemental composition and spectral characteristics of synthetic fulvic acids obtained from quercetin with natural fulvic acid allowed confirming their similarity. The content of basic acid groups in the structure of synthetic fulvic acid and their reduction capacity were calculated. In particular, the contents of carboxyl groups phenolic groups were $5.0 \pm 0.2 \text{ mmol g}^{-1}$ and $2.8 \pm 0.1 \text{ mmol g}^{-1}$, respectively; the reduction capacity being $3.07 \pm 0.2 \text{ mmol g}^{-1}$. The EPR spectrum of the FAH showed a sharp, narrow single line resonance at $g=2.0033$ with the line width of 3.6 Gauss and the concentration of paramagnetic centers of $4.8 \cdot 10^{16} \text{ spin g}^{-1}$. X-ray diffraction analysis of synthetic fulvic acids indicated their amorphous nature. SEM images of the dried preparations of synthetic fulvic acids showed the uniformity of the obtained powder particles.

Synthetic fulvic acids have the advantage over natural humic substances in terms of their reproducible properties because of their strict synthesis conditions. These substances inhibit *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The degree of activity or the sensitivity of test organisms amounts to $25 \mu\text{g mL}^{-1}$. Thus, the synthetic fulvic acid prepared by the method described here shows great promise as antimicrobial agents.

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КВЕРЦЕТИН ЯК ПРЕКУРСОР У СИНТЕЗІ АНАЛОГІВ ФУЛЬВОКИСЛОТ ТА ЇХ АНТИБАКТЕРІАЛЬНІ ВЛАСТИВОСТІ

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Запропоновано простий, швидкий та ефективний спосіб одержання синтетичних речовин із властивостями, подібними до природних гумінових речовин. Метод синтезу заснований на окисленні кверцетину молекулярним киснем у лужному середовищі з подальшим переведенням у форму кислоти шляхом пропускання через катіонообмінну колонку. Вивчення елементарного та функціонального складу, спектральних властивостей та окисно-відновних характеристик дозволило кваліфікувати одержаний продукт як синтетичну фульвокислоту. Встановлено посилені антибактеріальні властивості одержаного синтетичного продукту. Мінімальна концентрація інгібування для синтетичних фульвокислот з кверцетину становить 25 мкг/мл, що в 100 разів менше, ніж для природних гумінових речовин.

Ключові слова: кверцетин, прекурсор, синтетичні гумінові речовини, фульвокислоти, антибактеріальні властивості.

QUERCETIN AS A PRECURSOR IN THE SYNTHESIS OF ANALOGUES OF FULVIC ACIDS AND THEIR ANTIBACTERIAL PROPERTIESV.A. Litvin ^{a, *}, R.A. Njoh ^b^a The Bohdan Khmelnytsky National University of Cherkasy, Cherkasy, Ukraine^b Near East University, Nicosia, Cyprus

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A simple, fast and effective method for producing synthetic substances with properties similar to natural humic substances has been proposed. The synthesis method is based on the oxidation of quercetin by molecular oxygen in an alkaline medium, followed by conversion to the acid form by passing through a cation exchange column. Study of elemental and functional compositions, spectral properties (UV/Vis and IR range) and redox characteristics allowed qualifying the resulting product as a synthetic fulvic acid. The enhanced antibacterial properties of the obtained synthetic product were established. The minimum concentration of inhibition of synthetic fulvic acid derived from quercetin is 25 $\mu\text{g mL}^{-1}$, which is in 100 times less than for natural humic substances.

Keywords: quercetin; precursor; synthetic humic substance; fulvic acid; antibacterial properties.

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