Spectroscopic (FT-IR, FT-Raman, 1H, 13C NMR, UV/VIS), thermogravimetric and antimicrobial studies of Ca(II), Mn(II), Cu(II), Zn(II) and Cd(II) complexes of ferulic acid

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The molecular structure of Mn(II), Cu(II), Zn(II), Cd(II) and Ca(II) ferulates (4-hydroxy-3-methoxycinna-mates) was studied. The selected metal ferulates were synthesized. Their composition was established by means of elementary and thermogravimetric analysis. The following spectroscopic methods were used: infrared (FT-IR), Raman (FT-Raman), nuclear magnetic resonance (^{13}C, ^{1}H NMR) and ultraviolet–vis-ible (UV/VIS). On the basis of obtained results the electronic charge distribution in studied metal com-plexes in comparison with ferulic acid molecule was discussed. The microbiological study of ferulic acid and ferulates toward *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris* was done.

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Introduction

Nowadays there is a big social demand for natural, safe for human and environment food preservatives [1]. Because of the increase in the prevalence of allergies, colorectal and stomach cancers and cardiovascular diseases consumers are more and more concern about the quality of food that they eat [2,3]. Many natural antimicrobial compounds have been found in herbs, vegetables and fruits. Among them are cinnamic acid derivatives which beside antimicrobial activity possess other biological properties i.e. antioxidant, antifungal, antiseptic, antilisterial, and antitumor [4,5]. Ferulic acid (3-methoxy-4-hydroxycinnamic) may exist in two form cis and trans. This acid was detected in many plant and its properties are widely studied. Naz et al. isolated ferulic acid from ethanolic extract of the root bark of *Onosma hispidum* [6]. Tested extract possesses significant bioactivity *against corynebacteria, enterococci, staphylococci* and *streptococci*. Ferulic acid exhibited higher antimicrobial properties than vanillic acid. Ferulic acid is also a constituent of phenolic fractions of *Sutherlandia frutescens* and *Polyscias sambucifolia* which show substantial antimicrobial properties again Gram-positive bacteria [7]. Ferulic acid isolated from methanolic extract of *Rubus ulmifolius* was one of the most active phenolic compounds against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus niger* [8]. The antilisterial activity of ferulic acid was tested against five strains of *Listeria monocytogenes* [9]. The minimum inhibitory concentrations ranged between 0.20% and 0.27% (w/vol) and the compound was bactericidal at pH 4.5 and bacteriostatic at higher pH. Ferulic acid, because of its antioxidant and antifungal properties, has an application as copolymers in antimicrobial polymeric materials [10]. The antifungal activity was tested against *A. niger*, where a diminishment of fungal growth appears with the increment of the concentration, being completely inhibited at 10 mg/mL.

It is assumed that phenolic acids act as an antimicrobial compound both by the membrane and cytoplasmic mechanisms. Because phenolic acids are weak organic acids (the average of pK_a values in water are 3.89–4.44 for cinnamic acid and 4.2 for benzoic acid) their antimicrobial properties depend on the concentration of the undissociated molecules. It is suggested that undissociated forms of phenolic acids penetrate cell membrane by passive diffusion, dissociate in the cell and disturb the pH balance. The decrease in the cellular pH causes protein denaturation and the cell to die [11]. The second mechanism of action of phenolic acids consist in diffuse through the cytoplasmic membrane and increase its permeability [11]. This causes a leakage of bacterial cell constituents: proteins, nucleic acids, and inorganic ions such as potassium or phosphate. The mechanism of action of phenolic acids on the permeability of the cell membrane was described in the literature. For example, Campos at al. tested several phenolic acids for their effects on the cell membrane of *Oenococcus oeni* and *Lactobacillus hilgardii* [12]. The obtained results show that hydroxycinnamic acids (*p*-coumaric, caffeic and ferulic acids) cause greater ion leakages and higher proton influx than hydroxybenzoic acids (*p*-hydroxybenzoic, protocatechuic, gallic, vanillic, and syringic acids). *p*-Coumaric acid induces the strongest effect among the tested acids. Furthermore, the exposure of cells to phenolic acids brought about a significant increased the cell membrane permeability in both tested strains. The different effects of phenolic acids on membrane permeability were related to differences in their structure and lipophilic character.

Because phenolic acids are poorly soluble in water solution their limitation as an antibacterial compounds in water medium is obvious. In many cases the chemical and biological properties of phenolic compounds can be modified by formation of their complexes with metal ions. It depends on the kind of metal, structure of ligand and the type of metal–ligand interactions. The effect of metals on the properties of antimicrobial compounds has been widely studied since many years [13–15]. In work Ca(II), Mn(II), Cu(II), Zn(II) and Cd(II) complexes of ferulic acid were synthetized. Their composition was estimated on the basis of elementary and thermogravimetric methods. The spectroscopic (FT-IR, FT-Raman, UV/VIS, ^{13}C and ^{1}H NMR) and antimicrobial characteristic of these compounds toward *E. coli*, *Bacillus subtilis*, *C. albicans*, *P. aeruginosa*, *S. aureus* and *Proteus vulgaris* was done.

Materials and methods

The water solution of sodium ferulate ($C = 0.25\ mol/dm^3$) was added to the water solution of Cu(II), Cd(II), Ca(II) and Zn(II) chlorides ($C = 0.25\ mol/dm^3$) in a stoichiometric molar ratio 1:2. In order to obtain Mn(II) ferulate the concentration of sodium ferulate was $C = 0.5\ mol/dm^3$ and concentration of $MnCl_2$ was $C = 0.25\ mol/dm^3$. The precipitates of the complexes of Ca(II), Mn(II), Cu(II), Zn(II), and Cd(II) ferulates appeared. The precipitate was moved to paper dishes and rinsed with distilled water. The composition of complexes was established by the means of elementary analysis and thermogravimetric studies.

The FT-IR spectra of synthesized compounds were recorded with an Equinox 55 spectrometer and analyzed within the range 400–4000 cm^{-1}. Samples in the solid state were measured in KBr matrix pellets which were obtained with hydraulic press under 739 MPa pressure. The FT-Raman spectra of solid samples in capillary tubes were recorded in the range of 4000–400 cm^{-1} with a FT-Raman accessory of the Perkin Elmer System 2000. The resolution of spectrometer was 1 cm^{-1}. The DR 5000 Hach UV/VIS spectrophotometer was used in order to register electronic absorption spectra. The NMR spectra of DMSO-d6 saturated solutions were recorded with a Bruker Avance II 400 MHz unit at room temperature. TMS was used as an internal reference. The thermal stability and decomposition of the Ca(II), Mn(II), Cu(II) and Zn(II) ferulates was investigated with the aid of a Setsys 16/18 (Setaram) thermal analyzer recording TG/DSC/DTG curves. Samples (about 6 mg) were heated in ceramic crucible between 30–1000 °C (manganese(II) and zinc(II) compound) or 30–750 °C (calcium(II) and copper(II) complex) in flowing air atmosphere with a heating rate of 10 °C/min. The products of decomposition were postulated on the basis of the obtained TG curves.

The microbiological tests were conducted in the following way. The solution of ferulic acid and selected compounds: sodium, copper, cadmium and zinc ferulates were prepared by dissolving 0.10 g of compound in 10% water solution of DMSO, the final mass of solution was 10.00 g. Following species of microorganisms: *E. coli* (PCM 2268), *P. aeruginosa* (PCM 2270), *P. vulgaris* (PCM 2269), *S. aureus* (PCM 2267), *C. albicans* (PCM 2566) and *B. subtilis* (PCM 2021) were used for the test. The selected microorganisms were inoculated on broth medium and stored in 37°C (bacteria) or 25°C (fungus) for 24 h. Then 10.00 μl of prepared bacterial culture was added to 500.00 ml of sterile broth. Next, 4.75 μl of broth inoculated with various strains of microorganisms and 0.25 ml of ferulic acid and ferulate solutions was mixed in sterile test tube. The concentration of tested compound in the test tube was 0.10%. The samples were incubated in 35°C (bacteria) or 25°C (fungus). The increase in the number of colonies was estimated similarly after 24 and 48 h of incubation for at least four samples. The growth of tested cells was standardized using turbidimetry method by measuring optical density at 600 nm with a UV/VIS JASCO spectrophotometer. The obtained data were converted into the degree of growth inhibition according to the equation:

$$I = [(A_c - A_s)/A_c] \cdot 100\%, \text{where}$$

'*I*' it is a degree of growth inhibition of microorganism (%), 'A_c' is optical density of culture broth with the addition of 0.25 ml of 10% DMSO instead of tested compound, and A_s is the optical density of culture broth with the addition of sample solution. The negative values mean growth stimulation exhibited by tested compounds. Statistical calculations were performed using Microsoft Office Excel 2010 [16].

Results and discussion

Composition of ferulates

The results of elementary analysis for calcium, manganese, zinc, copper and cadmium ferulates are as follows. Calculated for $Ca_2C_{40}H_{42}O_{19}$: C 52.97% and H 4.67%; found: C 52.15% and H 4.90%; the general formula is $Ca(C_{10}H_9O_4)_2{\cdot}1.5H_2O$. Calculated for $Mn_2C_{40}H_{42}O_{19}$: C 51.30% and H 4.52%; found C 49.26% and H 4.34%; the general formula is $Mn(C_{10}H_9O_4)_2{\cdot}1.5H_2O$. Calculated for $Zn_2C_{40}H_{42}O_{19}$: C 50.17% and H 4.42%; found C 48.54 and H = 4.30%; the general formula is $Zn(C_{10}H_9O_4)_2{\cdot}1.5H_2O$. Calculated for $CuC_{20}H_{26}O_{12}$: C 46.02% and H 5.02%; found: C 45.78% and H 4.85%; the general formula is $Cu(C_{10}H_9O_4)_2{\cdot}4H_2O$. Calculated for $CdC_{20}H_{26}O_{12}$: C 42.09% and H 4.59%; found: C 42.19% and H 4.47%; the general formula is $Cd(C_{10}H_9O_4)_2{\cdot}4H_2O$.

The obtained compounds of the formulae: $Ca(C_{10}H_9O_4)_2{\cdot}1.5H_2O$, $Mn(C_{10}H_9O_4)_2{\cdot}1.5H_2O$, $Cu(C_{10}H_9O_4)_2{\cdot}4H_2O$ and $Zn(C_{10}H_9O_4)_2{\cdot}1.5H_2O$ have been investigated by TG-DSC thermal method in order to estimate the influence of the temperature on the structure of these complexes and determine the endothermic or exothermic effects connected with dehydration and oxidation. In all cases, heating of the hydrated complexes above 30 °C resulting in dehydration process, which as can be seen from Fig. 1 occurs only in one stage. The first distinct weight loss corresponds to the removal of all water molecules takes place in the temperature range 30–135, 30–145 and 30–150 °C for Ca/Cu, Mn and Zn complexes, respectively (Table 1). Weak endothermic effects seen at the DCS curves at about 80, 85, 105 and 90 °C for complexes of Ca, Mn, Cu and Zn, respectively accompany these reactions. The dehydration process occurring at lower values of temperature is connected with the release of weakly bonded water molecules. It is possible that these non-coordinated water molecules were in the inner coordination sphere of metal ions being hydrogen bonded with the organic ligand.

The dehydration process yield in formation of anhydrous $M(C_{10}H_9O_4)_2$ (where M = Ca, Mn, Cu or Zn) compounds which are not thermally stable and immediately decompose via intermediate forms to solid residues, namely to the suitable metal oxides: CaO, MnO_2, CuO and ZnO. In each instant, as follows from Fig. 1, the stage of thermal decomposition of anhydrous form of complexes

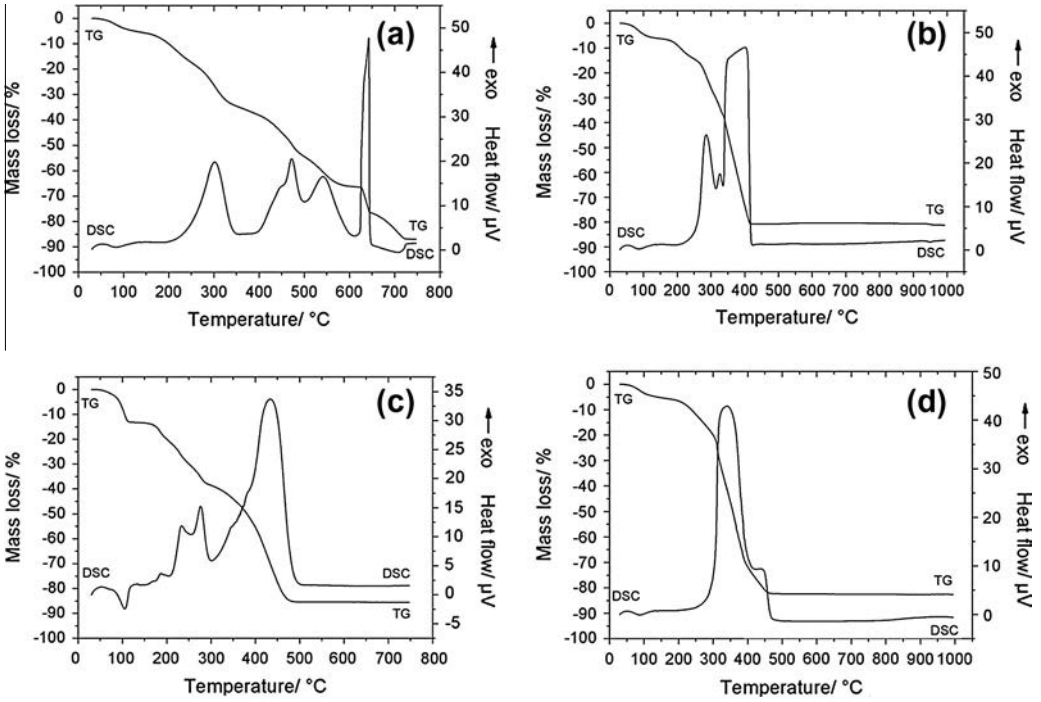

Fig. 1. TG-DSC plots of thermal decomposition of: (a) $Ca(C_{10}H_9O_4)_2{\cdot}1.5H_2O$; (b) $Mn(C_{10}H_9O_4)_2{\cdot}1.5H_2O$; (c) $Cu(C_{10}H_9O_4)_2{\cdot}4H_2O$ and (d) $Zn(C_{10}H_9O_4)_2{\cdot}1.5H_2O$.

Table 1
Results of thermal decomposition of calcium, manganese, copper and zinc ferulates in air atmosphere; $L = (C_{10}H_9O_4)^-$.

Complex	T_1 (°C)	T^*_{endo}	Mass loss (%)		Intermediate solid product	T_2 (°C)	Residue (%)		Residue
			Found	Calc.			Found	Calc.	
$CaL_2{\cdot}1.5H_2O$	30–135	80	5.64	5.96	CaL_2	135–740	13.13	12.36	CaO
$MnL_2{\cdot}1.5H_2O$	30–145	85	6.15	5.77	MnL_2	145–440	19.06	18.57	MnO_2
$CuL_2{\cdot}4H_2O$	30–135	105	13.32	13.80	CuL_2	135–510	14.56	15.24	CuO
$ZnL_2{\cdot}1.5H_2O$	30–150	90	5.28	5.64	ZnL_2	150–500	17.98	17.02	ZnO

T_1 – Temperature range of dehydration.
T_2 – Temperature range of degradation of anhydrous complexes to suitable oxides.
T^*_{endo} – Very weak endothermic effect.

Table 2
Wavenumbers, intensities and assignments of selected bands occurring in the FT-IR and FT-Raman spectra of ferulic acid and zinc(II), calcium, cadmium(II), manganese(II) and copper(II) ferulates.

Ferulic acid	Zn(II) ferulate		Ca(II) ferulate		Cd(II) ferulate		Mn(II) ferulate		Cu(II) ferulate	Assignment	According to Varsányi [17]
FT-IR	FT-IR	Raman	IR	Raman	IR	Raman	IR	Raman	IR		
3437 s[a]	3426 s		3362 m		3418 s		3426 s		3219 s	$\nu(OH)_{ar}$	
3078 m										$\nu(OH)_{COOH}$	
2970 m										$\nu_{as}(CH_3)$	
2942 m	2938 w		2938 w		2938 m	2944 vw	2936 s		2940 m	$\nu_s(CH_3)$	
1692 vs 1668 s										$\nu(C{=}O)$	
1620 vs	1638 m	1638 vw	1640 s	1640 vw	1636 s	1642 vw	1634 s	1637 vw	1641 s	$\nu(C{=}C)_{-c=c-}$	
1601 vs	1595 m	1601 vw	1595 m	1601 vw	1595 s	1602 vw	1597 s	1597 vw	1574 s	$\nu(CC)_{ar}$	8b
1552 w	1553 w		1536 s		1554 sh	1544 vw	1557 s		1550 sh	$\nu(CC)_{ar}$	8a
1516 vs										$\nu(CC)_{ar}$	19a
	1510 s	1508 vw	1514 m		1514 vs		1510 vs		1516 vs	$\nu_{as}(COO^-)$	
1466 s	1450 m	1452 vw	1449 w	1451 vw	1449 s	1449 vw	1450 s	1451 vw	1452 vs	$\delta_{as}(CH_3)$	
1433 s	1425 w	1431 vw	1427 w	1430 vw	1427 s	1427 vw	1425 vs	1429 vw	1433 s	$\nu(CC)_{ar} + \delta_s(CH_3) + \beta(CH)_{-c=c-} + \beta(CH)_{ar} + \beta(OH)_{ar}$	19b
	1404 s	1407 vw	1404 vs		1399 vs	1399 vw	1402 vs	1403 vw	1406 vs	$\nu_s(COO^-)$	
1414 m										$\beta(OH)_{ar} + \beta(CH)_{ar}$	
1379 m										$\beta(OH)_{COOH} + \beta(CH)_{-c=c-}$	3
1290 s										$\nu(C{-}O) + \beta(CH)_{-c=c-} + \beta(CH)_{ar}$	
1277 vs	1273 s	1274 vw	1275 m	1274 vw	1273 vs	1278 vw	1267 vs	1273 vw	1275 vs	$\beta(CH)_{-c=c-} + \beta(CH)_{ar} + \nu(C{-}O)$	
1323 s	1217 w		1213 w		1217 s				1237 s	$\beta(CH) + \rho(CH_3) + \beta(OH)_{ar}$	
1206 vs										$\beta(CH) + \rho(CH_3) + \beta(OH)_{ar}$	13
1113 m	1126 s	1128 vw	1125 m	1127 vw	1126 s	1121 vw	1124 s	1127 vw	1125 s	$\beta(CH)$	
1036 m	1030 s	1034 vw	1030 m		1030 s		1030 s		1038 m	$\nu O{-}(CH_3)$	
972 w	982 m	981 vw	980 m		980 m		982 m		966 m	$\gamma(CH)_{-c=c-}$	
945 s										$\gamma(OH)_{COOH}$	
853 m	853 m		853 m		853 m		860 m	978 vw	866 w	$\gamma(CH)_{ar}$	
	822 m	814 vw	820 m		822 m	821 vw	816 m	813 vw	816 m	$\beta_s(COO^-)$	
804 m										$\beta(C{=}O)$	
	712 w	714 vw	712 w		718 m	714 vw	723 w		708 m	$\gamma_s(COO^-)$	
600 m	590 m		603 w		584 m	582 vw	604 m			$\alpha(CCC)$	6a
575 m										$\gamma(C{=}O)$	
	577 m		575 m		577 m	574 vw	575 m		575 m	$\beta_{as}(COO^-)$	
521 m	523 m		528 w		517 m		527 w		511 w	$\beta\ O{-}(CH_3)$	
446	460 m		461 w		438 m		463 w		474 vw	$\Phi(CC)$	16a

[a] Symbols mean: vs – very strong, s – strong, m – medium, w – weak, vw – very weak.

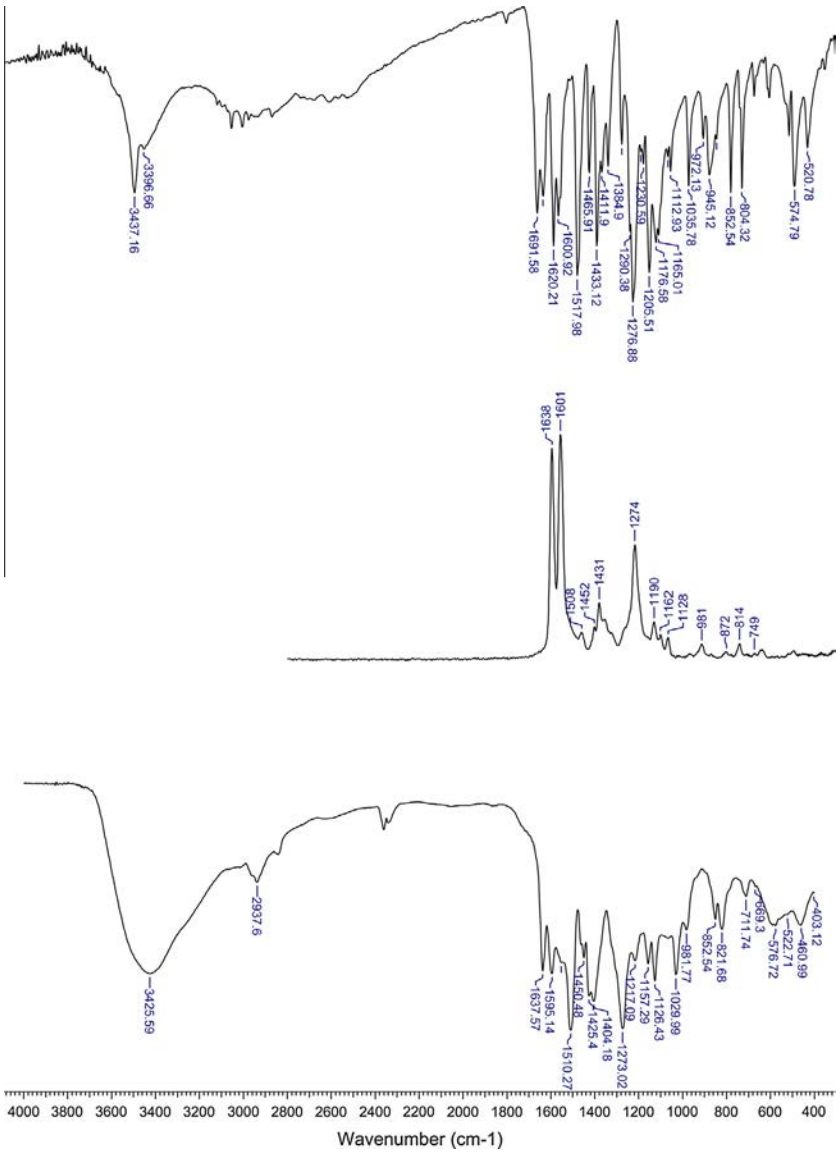

Fig. 2. The FT-IR spectrum of ferulic acid (a), and FT-Raman (b) and FT-IR (c) spectra of zinc ferulate.

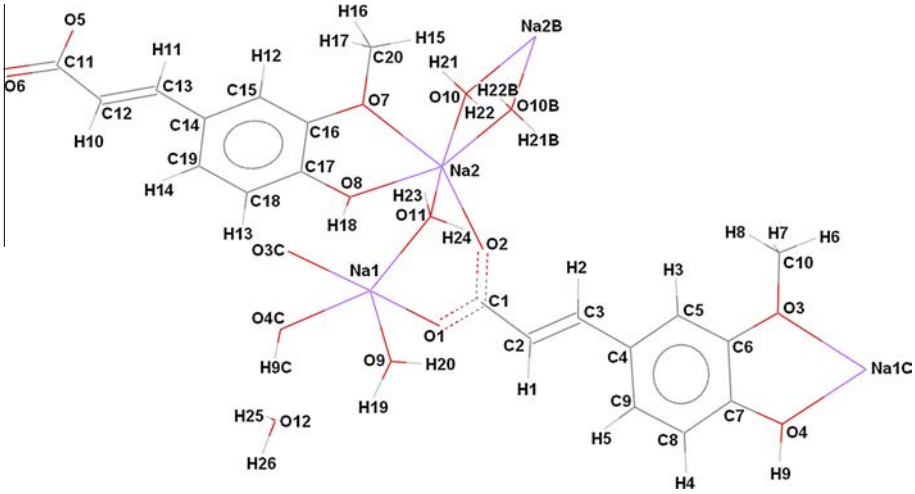

Fig. 3. The crystal structure of sodium ferulate; on the basis of [18].

Table 3
The ^{13}C NMR and ^{1}H NMR chemical shifts (ppm) for ferulic acid and metal ferulates (atom numbering in Fig. 4).

Atom position	Ferulic acid	Calcium ferulate	Cadmium(II) ferulate	Copper(II) ferulate	Zinc(II) ferulate
C1	124.62	119.12	126.53	124.14	126.17
C2	111.00	110.59	110.71	113.15	111.02
C3	151.40	147.83	148.51	149.89	147.89
C4	149.40	147.44	147.88	149.71	147.70
C5	117.78	115.53	119.39	117.98	119.52
C6	122.55	119.05	121.87	123.45	122.35
C7	148.95	121.45	141.65	130.34	136.69
C8	117.58	115.38	115.62	113.15	111.02
C9	169.94	–	173.67	–	–
C10	57.42	55.56	55.66	55.53	55.67
H_A	12.11	–	–	–[a]	–
H_B	6.37	6.27	6.39		6.34
H_C	7.50	7.30	7.34		7.40
H_D	7.27	7.10	7.18		7.23
H_E	3.82	3.76	3.81		3.79
H_G	6.79	6.75	6.76		6.75
H_H	7.08	6.93	6.96		7.04

[a] Decomposition during measurement.

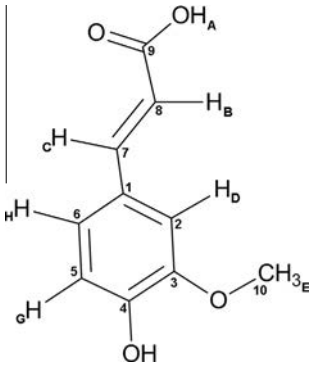

Fig. 4. Atom numbering of ferulic acid molecule.

proceeds in dissimilar pathway. The tremendous contrast is concerned with the number of degradation steps of ferulate ligand that is four – for Ca and Cu complex, three – for Mn compound or two – for Zn complex. In oxidative air atmosphere degradation of ferulate ligand is accompanied by strong exothermic effects connected with burning of organic part of compounds. Thermal data show that the temperature of aforementioned oxides formation is in the range 440–740 °C (Table 1).

FT-IR, FT-Raman, ^{13}C and ^{1}H NMR, and UV/VIS studies of ferulates

The wavenumbers, intensities and assignments of bands occurring in the FT-IR and FT-Raman spectra of ferulic acid and studied ferulates are shown in Table 2. The spectra of metal ferulates and ferulic acid are in Fig. 2. The symbols used were given by Varsányi and notice the following: ν stands for stretching vibrations, β for in-plane bending modes, γ for out-of-plane bending modes, φ(CCC) for the aromatic ring out-of-plane bending modes, α(CCC) for the aromatic ring in-plane bending modes, $\delta(CH_3)$ for the methyl group rocking deformations [17].

The ferulic acid spectrum shows a characteristic band of stretching vibrations of the carbonyl group C=O: ν(C=O) 1692–1668 cm^{-1}, out-of-plane vibrations of carboxylic group: $\gamma(OH)_{COOH}$ 945 cm^{-1}, γ(C=O) 575 cm^{-1}, and a band of stretching vibrations of the hydroxyl group $\nu(OH)_{COOH}$ (3078 cm^{-1}). The spectra of metal complexes do not show a characteristic band of stretching vibration of these bands which prove that the synthesis was done correctly. Replacement of the carboxylic group hydrogen with a metal ion causes a breakdown of the intermolecular hydrogen bonding and

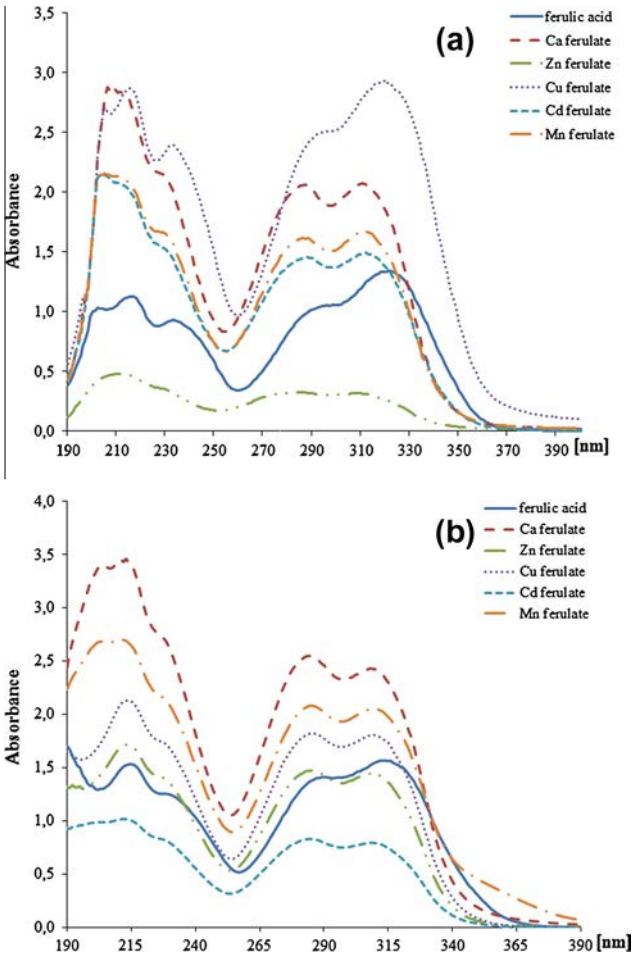

Fig. 5. UV/VIS spectra of ferulic acid and ferulates recorded in (a) methanol and (b) water.

therefore the characteristic changes in the IR and Raman spectra of the metal ferulates in comparison with the spectrum of acid appeared. Namely, in the spectra of complexes the bands assigned to carboxylic group vibrations disappeared, peaks derived from carboxylate anion vibration occurred and changes in the position and intensities of some aromatic bands were also reported.

In the spectrum of acid there is a band at 3437 cm^{-1} assigned to the stretching $\nu(OH)_{ar}$ vibrations of the substituent in the ring. In the spectrum of metal ferulates this band is shifted toward lower wavenumber (3426–3219 cm^{-1}). In Fig. 3 one can see that in sodium ferulate the sodium cation is coordinated also by oxygen from OH substituent in the ring [18]. The participation of substituent in metal coordination decreases the strength of the O—H bond. The movement of $\nu(OH)_{ar}$ toward lower wavenumbers in the spectrum of metal complexes suggests a decrease in the electronic charge density of the O—H bond and weakness of this bond compared with acid molecule. Therefore the participation of hydroxyl substituent in metal binding is possible.

In the region of 3000–2900 cm^{-1} there are bands assigned to the stretching O—CH_3 vibrations. At about 1630 cm^{-1} the band that comes from stretching vibrations of the double bond between a ring and a carboxylic/carboxylate group $\nu(C{=}C)_{-c=c-}$ is present (1638 cm^{-1}, 1640 cm^{-1}, 1636 cm^{-1}, 1634 cm^{-1}, 1641 cm^{-1} and 1620 cm^{-1}, for Zn ferulate, Ca ferulate, Cd ferulate, Mn ferulate, Cu ferulate and ferulic acid, respectively). This band is shifted toward lower wavenumbers in the spectrum of acid compared with complexes. It suggests an increase in the electronic charge density around double bond —C=C— in ferulates comparing to ferulic acid. In the region of 1602–1425 cm^{-1} are bands derived from stretching vibrations of the aromatic ring. These bands are placed at about similar wavenumbers both in the spectrum of acid and complexes. The absence of these bands in the spectra of metal ferulates, caused by the weakening of the bonds, or the decrease in their intensity is considered as an evidence of an increase in the perturbation of the aromatic system of the ring. In the region of 1470–850 cm^{-1} bands derived from bending modes and rocking deformations of the C—H, O—H and CH_3 bonds are present. The slight changes in the position of peaks assigned to O—CH_3 vibrations are caused by the formation of metal complex. The wavenumbers for $\delta_{as}(CH_3)$ are following: 1450 cm^{-1}, 1449 cm^{-1}, 1449 cm^{-1}, 1450 cm^{-1}, 1452 cm^{-1}, and 1466 cm^{-1} for Zn ferulate, Ca ferulate, Cd ferulate, Mn ferulate, and ferulic acid, respectively. The aromatic ring in-plane bending modes are at similar wavenumbers in the spectra of ferulic acid and its complexes (at about 600 cm^{-1}).

In Table 3 the chemical shifts from ^{13}C and ^{1}H NMR spectra of ferulic acid and metal ferulates are gathered. The differences between the values of chemical shifts from NMR spectra of ferulates and acid show that metal ions influence the electronic charge distribution around particular carbon and hydrogen atoms. Substitution of metal ion in the carboxylic group of ligand causes an increase in the electronic charge density around C3, C4, C7, C8 and C10, and a decrease around atoms C1 (except Ca ferulate) and C5 (except Ca ferulate) (Fig. 4) comparing to acid molecule. The most distinct changes concern the electronic charge distribution in the region of the double bond between carboxylate group and the ring. The differences between the values of chemical shifts of C7 and C8 atoms are: 31.37 ppm for acid, 26.03 ppm for cadmium ferulate, 14.34 ppm for zinc ferulate, 6.89 ppm for copper ferulate, and 6.07 ppm for calcium ferulate. It means that the difference in the electronic charges gathered on C7 and C8 atoms becomes more equal along the series: Ca ferulate → Cu ferulate → Zn ferulate → Cd ferulate → ferulic acid. In case of ^{1}H NMR spectra, the most distinct differences between the spectra of ferulic acid and ferulates concern the chemical shifts of H_B, H_C and H_D protons (especially Ca complex). The NMR data show that the influence of metal cations on the molecular structure of ferulic acid may be described as a short-range effect.

The UV/VIS absorption spectra of ferulic acid and ferulates were recorded in methanol and water and displayed in Fig. 5 and Table 4. The bands at 200, 210 and 230 nm correspond to $\pi \rightarrow \pi^*$ transitions, where the bands located at higher nm results from $n \rightarrow \pi^*$ transitions. The location of some bands depends on the kind of solvent, i.e. bands no. 3 and 5. The spectra of ferulates show differences between each other when they are recorded in methanol. The UV/VIS spectra of ferulates recorded in water do not exhibit significant differences.

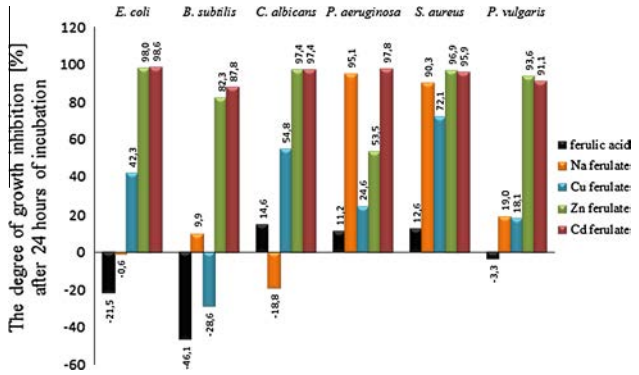

Fig. 6. The degree of growth inhibition (%) of *E. coli*, *B. subtilis*, *C. albicans*, *P. aeruginosa*, *S. aureus* and *P. vulgaris* exhibited by tested compounds after 24 h of incubation. The negative values mean growth stimulation. The mean values of the degree of growth inhibition are depicted in the plot.

Table 4
Band maximum from UV/VIS spectra of methanol and water solution of ferulic acid and ferulates.

Compound	Band 1	Band 2	Band 3	Band 4	Band 5
In methanol					
Ferulic acid	202	216	233	291	321
Ca ferulate	206	210	224	287	311
Zn ferulate		211	227	284	309
Cu ferulate	205	215	232	290	320
Cd ferulate	204	213	225	288	312
Mn ferulate	205	210	226	288	311
In water					
Ferulic acid		215	228	290	313
Ca ferulate	205	211	225	284	309
Zn ferulate		213	226	285	309
Cu ferulate		213	226	285	309
Cd ferulate	202	212	226	285	309
Mn ferulate	204	211	225	285	309

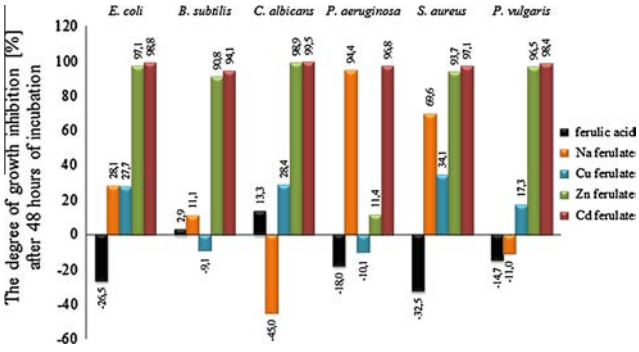

Fig. 7. The degree of growth inhibition (%) of *E. coli*, *B. subtilis*, *C. albicans*, *P. aeruginosa*, *S. aureus* and *P. vulgaris* exhibited by tested compounds after 48 h of incubation. The negative values mean growth stimulation. The mean values of the degree of growth inhibition are depicted in the plot.

Antimicrobial activity of ferulates

The results of microbiological analysis are presented in Figs. 6 and 7. Selected ferulates were chosen in this study, i.e. transition metal complexes with covalent bond between central ion and carboxylate group and sodium ferulate with ionic type of bonding. Cadmium ferulate has been deliberately included in the study as

a compound with potentially very strong antimicrobial properties, which could be applied on the highly resistant cell lines. Because the 10% solution of DMSO was used as a solvent, it was also screened against all organisms and no activity was found. The tested compounds show different antimicrobiological activity. Ferulic acid does not inhibit the growth of *E. coli*, *B. subtilis*, *C. albicans*, *P. aeruginosa*, *S. aureus* and *P. vulgaris* at all. The highest degree of growth inhibition was obtained in case of *C. albicans* at quite low level, i.e. I_{24h} = 14.6% and I_{48h} = 13.3% after 24 and 48 h of incubation, respectively. After 48 h of incubation ferulic acid stimulates the growth of microbial colony. Only in case of *B. subtilis* antimicrobial activity of ferulic acid increases after 48 h of incubations but still rises to a very low level of *I*, i.e. $I_{24h} = -46.1\%$ and I_{48h} = 2.9% after 24 and 48 h of incubation, respectively.

Sodium, copper, zinc and cadmium ferulates possess relatively higher antimicrobial properties comparing with ferulic acid. Namely, Na ferulate inhibits the growth of *P. aeruginosa* (I_{24h} = 95.1% and I_{48h} = 94.4%) and to a lesser degree the growth of *S. aureus* (I_{24h} = 90.3% and I_{48h} = 69.6%). Na ferulate clearly shows a lack of antimicrobial activity toward *C. albicans* ($I_{24h} = -18.8\%$ and $I_{48h} = -45.0\%$) and moderate properties in case of *E. coli* ($I_{24h} = -0.6\%$ and $I_{48h} = -28.1\%$), *B. subtilis* (I_{24h} = 9.9% and I_{48h} = 11.1%) and *P. vulgaris* (only after 24 h of incubation I_{24} = 19.0%). Cu ferulate inhibits the growth of *E. coli* and *C. albicans* to a certain degree (i.e. I_{24h} = 42.3% and I_{24h} = 54.8%, respectively) as well as the growth of *S. aureus* to a higher degree (I_{24h} = 72.1%), but the antimicrobial properties of this compound decrease after 48 h of incubation. The highest antimicrobial activities in this study possess Zn and Cd ferulates in case of all tested microorganisms at the level of 82–99% (I) and the properties do not decrease after 48 h of incubation. Only for *P. aeruginosa* the obtained degree of inhibition is lower and equals I_{24h} = 53.5% and I_{48h} = 11.4%.

Higher antimicrobial activity of ferulates compared with ferulic acid suggests that the ionic form of ligand (in case of sodium ferulate) or coordination compounds with transition metals are the dominant forms which show effective antimicrobial properties. Generally, the antimicrobial activity of tested compounds increases in the following order: ferulic acid < sodium ferulate < transition metal ferulates. The biological activity of tested compounds is significantly increased on coordination. What is the reason of higher antimicrobial property of transition metal complexes than sodium salt or ligand? One of the most important factors determining the antimicrobial activity of substance is lipophilicity. The outer lipid membrane of cell favors the passage of lipid soluble compounds. According to Tweedy's chelation theory coordination reduces the polarity of metal ion due to partial sharing of the positive charge of metal ion with the donor groups and possible delocalization of the π-electron charge over the chelate ring system [19–21]. In such a way coordination compounds possess enhance lipophilic character of the central metal atom what makes them easier to penetrate the lipid layers of the cell membrane and interact with components of the bacterial or fungal cell.

Conclusion

Ca(II), Mn(II), Cu(II), Zn(II) and Cd(II) complexes of ferulic acid were synthetized in the form of solid residue. Their composition was establish by means of elementary and thermogravimetric analysis. The formation of metal complexes causes changes in the electronic charge distribution within the molecules compared with acid. The increase in the electronic charge density around atoms C3, C4, C7, C8 and C10, and a decrease around atoms C1 and C5 in ferulate molecules comparing with ligand was observed. The most distinct changes in the electronic charge distribution concern the carbon atoms of the double bond between carboxylate group and the ring. The hydroxy and methoxy substituents take part in metal binding. The difference in the electronic charges gathered on C7 and C8 atoms becomes more equal along the series: Ca ferulate → Cu ferulate → Zn ferulate → Cd ferulate → ferulic acid.

Coordination and chelation cause that metal complexes are more powerful antimicrobial compounds than ligand. Zinc and cadmium ferulates possess the strongest antimicrobial properties toward tested microorganisms. Cu ferulate inhibits the growth of *E. coli*, *C. albicans* and *S. aureus*, but to a lesser degree than Zn and Cd compounds. Na ferulate is active toward *P. aeruginosa* and *S. aureus*.

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References

[1] Soon-Mi Shim, Sun Hee Seo, Youngja Lee, Gui-Im Moon, Min-Shik Kim, Ju-Hee Park, Food Control 22 (2011) 1054–1060.
[2] S.H. Magnússon, H. Gunnlaugsdóttir, H. van Loveren, F. Holm, N. Kalogeras, O. Leino, J.M. Luteijn, G. Odekerken, M.V. Pohjola, M.J. Tijhuis, J.T. Tuomisto, Ř. Ueland, B.C. White, H. Verhagen, Food Chem. Toxicol. 50 (2012) 33–39.
[3] K. Senior, Mol. Med. Today 3 (1997) 103–107.
[4] P. De, M. Baltas, F. Bedos-Belval, Curr. Med. Chem. 18 (2011) 1672–1703.
[5] P. Sharma, J. Chem. Pharm. Res. 3 (2011) 403–423.
[6] S. Naz, S. Ahmad, S.A. Rasool, S.A. Sayeeda, R. Siddiqi, Microbiol. Res. 161 (2006) 43–48.
[7] M.A. Fernández, M.D. Garcia, M.T. Sáenz, J. Ethnopharmacol. 53 (1996) 11–14.
[8] L. Panizzi, C. Caponi, S. Catalano, P.L. Cioni, I. Morelli, J. Ethnopharmacol. 79 (2002) 165–168.
[9] A. Wen, P. Delaquis, K. Stanich, P. Toivonen, Food Microbiol. 20 (2003) 305–311.
[10] F. Iemma, F. Puoci, M. Curcio, O.I. Parisi, G. Cirillo, U.G. Spizzirri, N. Picci, J. Appl. Polym. Sci. 115 (2010) 784–789.
[11] M.D. Johnston, G.W. Hanlon, S.P. Denyer, R.J.W. Lambert, J. Appl. Microbiol. 94 (2003) 1015–1023.
[12] F.M. Campos, J.A. Couto, A.R. Figueiredo, I.V. Tóth, A.O. Rangel, T.A. Hogg, Int. J. Food Microbiol. 31 (2009) 144–151.
[13] E.D. Weinberg, Bacteriol. Rev. 21 (1957) 46–68.
[14] D. Scharfenberg-Pfeiffer, M. Czugler, Pharmazie 46 (1991) 781–783.
[15] G. Wilkinson, Comprehensive Coordination Chemistry. The Synthesis, Reactions, Properties and Application of Coordination Compounds. Vol. 5. Late Transition Elements, Pergamon Press, 1987.
[16] Microsoft Office Excel, Microsoft, 2010.
[17] Varsányi, Assignments for Vibrational Spectra of 700 Benzene Derivatives, Akademia Kiado, Budapest, 1973.
[18] A. Kula, L. Mazur, Z. Rzaczynska, J. Coord. Chem. 60 (2007) 843–850.
[19] S.A. Patil, V.H. Naik, A.D. Kulkarni, P.S. Badami, Spectrochim. Acta Part A 75 (2010) 347–354.
[20] G. Krishnan, S. Suprabha, Int. J. Chem. Tech. Res. 4 (2012) 484–492.
[21] K. Shanker, R. Rohini, V. Ravinder, P.M. Reddy, Y.-P. Ho, Spectrochim. Acta Part A 73 (2009) 205–211.