

UDC 541.49:546.47:546.73:546.56:541.6

**DIFFERENT PERIPHERAL SUBSTITUTED PHTHALOCYANINES:
SYNTHESIS, CHARACTERIZATION, AGGREGATION BEHAVIOR,
ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY****M. Çelebi¹, M.S. Ağirtaş¹, A. Dundar²**¹*Department of Chemistry, Faculty of Science, Yüzüncü Yıl University, 65080, Van, Turkey*
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In this study, a novel phthalonitrile, 4-chloro-5-(2-((2-hydroxyethyl)(*p*-tolyl)amino)ethoxy)phthalonitrile (**3**), and its metallophthalocyanine derivatives (**4—6**) are prepared by cyclo-tetramerization with appropriate metal salts in dimethylformamide. The newly prepared compounds have been characterized by several spectroscopic techniques. All compounds are evaluated for their antioxidant and antibacterial potential. For the antioxidant studies, three tests are applied; DPPH (2,2-diphenyl-1-picrylhydrazylradical) scavenging, metal chelating and reducing power activity. Compound **4** exhibits the best DPPH scavenging activity as 35.2 % at 100 mg/L concentration. The metal chelating activities of compounds **3** and **4** are 69.7 % and 56.4 %, respectively. Reducing power activities of compounds **3** and **4** are higher than α -tocopherol which is used as positive control. All compounds show moderate antibacterial activity when compared to the standard antibiotics, amikacin and tetracycline.

DOI: 10.15372/JSC20150828

Keywords: synthesis, metal, phthalocyanines, aggregation, antioxidant, antibacterial activity.**INTRODUCTION**

The synthesis of the peripherally substituted phthalocyanines has attracted considerable attention due to their electronic properties and chemical structure [1, 2]. The unique properties of phthalocyanines have encouraged researchers to synthesize new derivatives with certain properties required for applications such as therapeutic medicine, photodynamic therapy, materials science, catalytic therapy and antioxidant potentials [3—5]. For this purpose, many applications of phthalocyanines in different fields, including dyes and pigments [6], semiconductors [7], optical data storage [8], photosensitizers [9], liquid crystals [10], nonlinear optics [11], gas sensors [12], catalysis [13] and Langmuir—Blodgett films [14] have been found. However, there are some notable drawbacks for the use of phthalocyanines, like poor solubility in a wide range of solvents and the aggregation tendency to form aggregates in solution [15]. To overcome these drawbacks, starting materials with suitable substituents must be chosen to synthesize new phthalocyanine derivatives. For this purpose, 2,2'-(4-methylphenylimino)diethanol can be chosen as a starting material since it is soluble in organic solvents and does not have a tendency to form aggregates. It has a good potential to evaluate the antioxidant and antibacterial activities. The most current research on antioxidant action focuses on phenolic compounds and phthalocyanine derivatives. The antioxidants are show the functions as singlet-triplet oxygen quencher, free radical scavengers, peroxide decomposers, enzyme inhibitors and synergists

[16, 17]. It is believed that antioxidants play a very important role in the body defense system against reactive oxygen species.

In this study, we describe the synthesis and characterization of phthalocyanines bearing tetra 4-chloro-5-(2-((2-hydroxyethyl)(*p*-tolyl)amino)ethoxy) substituents in the peripheral positions. We also report the results of a comprehensive investigation of the concentration effects on the aggregation properties of phthalocyanine derivatives in THF. Additionally, the antioxidant and antimicrobial activities of the phthalocyanines have been investigated by using antioxidant assays and disc diffusion method.

EXPERIMENTAL

General. The starting material, 4,5-dichlorophthalonitrile, was synthesized by the literature method [18]. Other chemicals were purchased from Sigma, Aldrich etc. and used without purification. The solvents were purified according to the standard procedures [19] and stored over molecular sieves (4 Å). All reactions were carried out under dry nitrogen. The purity of the novel products was tested in each step by thin layer chromatography. Melting points were measured on an electrothermal apparatus. Electronic spectra were recorded on a Hitachi U-2900 Spectrophotometer (Van YYU, Central Laboratory, Turkey). Routine IR spectra were recorded on a Thermo Scientific FTIR (ATR sampling accessory) spectrophotometer (Van YYU, Central Laboratory, Turkey). Mass spectra were recorded on an AB Sciex 3200 Q Trap LC-MS/MS spectrometry and MALDI (matrix assisted laser desorption ionization) BRUKER Microflex LT using 2,5-dihydroxybenzoic acid as matrix. ¹H NMR spectra were recorded on a Bruker 300 MHz spectrometer (Malatya IBTAM, Central Laboratory, Turkey).

4-Chloro-5-(2-((2-hydroxyethyl)(*p*-tolyl)amino)ethoxy)phthalonitrile (3). A mixture of 2,2'-(4-methylphenylimino)diethanol **2** (0.976 g, 5 mmol) and 4,5-dichlorophthalonitrile **1** (0.985 g, 5 mmol) in 25 mL dimethylformamide (DMF) was stirred at room temperature under nitrogen atmosphere. After stirring for 15 min, K₂CO₃ (2.5 g, 18 mmol) was added into the mixture over a period of 2 h. After stirring for a further 24 h, the reaction mixture was poured into water (200 mL) and extracted with CH₂Cl₂. The crude product was washed first with 10 % NaHCO₃ and then with water and dried over anhydrous sodium sulfate. The solution was evaporated to dryness. The product is soluble in THF, ethanol, acetonitrile, acetone, ethyl acetate, CH₂Cl₂, CHCl₃, DMF and DMSO. Yield: 1.128 g (64 %). MP: 117 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ, ppm: 8.34—6.67 (Ar—H), 3.54 (OH), 4.38—3.78 (aliphatic groups), 2.50 (CH₃). IR spectrum (cm⁻¹): 3276 (OH), 3085—3015 (Ar—CH), 2923 (CH₃), 2238 (CN), 1617, 1575, 1257, 1221, 1142, 1044, 914, 802, 683 and 529. Anal. Calc. for C₁₉H₁₈ClN₃O₂ (%): C 64.13, H 5.10, N 11.81. Found (%): C 64.21; H 5.18; N 11.74. MS (ES), *m/z* = 355 [M]⁺.

Synthesis of zinc(II) phthalocyanine (4). A mixture of 4-chloro-5-(2-((2-hydroxyethyl)(*p*-tolyl)amino)ethoxy)phthalonitrile **3** (0.142 g, 0.40 mmol) and ZnCl₂ (0.020 g) were dissolved in dimethylformamide (2 mL) under nitrogen in the presence of DBU. The mixture was refluxed under stirring for 24 h. After cooled down the crude product was precipitated by adding water. The precipitate was filtered, washed with cold and hot ethanol, methanol several times. Then, it was extracted with THF and dried. It was then purified by column chromatography on silicagel with THF as eluent. The product is soluble in CHCl₃, CH₂Cl₂, THF, DMF and DMSO. The yield was 0.055 g (37 %). ¹H NMR (DMSO-*d*₆) δ, ppm: 8.98—6.64 (Ar—H), 3.47 (OH), 4.83—3.85 (aliphatic groups), 2.50—2.19 (CH₃). UV-Vis (THF) λ_{max} (log ε): 670 (4.73), 612 (4.38), 346 (4.66). IR spectrum (cm⁻¹): 3328 (OH), 3062 (Ar—CH), 2910—2848 (CH₃), 1601, 1517, 1257, 1181, 1085, 1042, 882, 741, 655 and 507. Calc. for C₇₆H₇₂Cl₄N₁₂O₈Zn (%): C 61.32, H 4.87, N 11.29. Found (%): C 61.39, H 4.79, N 11.22. MS: *m/z* 1488 [M]⁺.

Synthesis of cobalt(II) phthalocyanine (5). The procedure is similar to the synthesis of **4** from **3**, except using CoCl₂. The product is soluble in DMF, THF, CHCl₃, CH₂Cl₂ and DMSO. The yield was 0.065 g (44 %). UV-Vis (THF) λ_{max} (log ε): 660 (4.71), 320 (4.69). IR spectrum (cm⁻¹): 3330 (OH), 3073 (Ar—CH), 2921—2859 (CH₃), 1605, 1518, 1257, 1227, 1088, 1044, 830, 1797, 747, and 503. Calc. for C₇₆H₇₂Cl₄N₁₂O₈Co (%): C 61.58, H 4.90, N 11.34. Found (%): C 61.54, H 4.96, N 11.42. MS: *m/z* 1482.2 [M]⁺.

Synthesis of copper(II) phthalocyanine (6). The procedure is similar to the synthesis of **4** from **3**, except using CuCl_2 . The product is soluble in CH_2Cl_2 , CHCl_3 , DMF, THF and DMSO. The yield was 0.040 g (27 %). UV-Vis (THF) λ_{max} (log ϵ): 676 (4.69), 616 (4.38), 340 (4.60). IR spectrum (cm^{-1}): 3330 (OH), 3067 (Ar—CH), 2919—2853 (CH_3), 1605, 1534, 1261, 1232, 1071, 1048, 799, 742, 717, and 521. Calc. for $\text{C}_{76}\text{H}_{72}\text{Cl}_4\text{N}_{12}\text{O}_8\text{Cu}$ (%): C 61.39, H 4.88, N 11.30. Found (%): C 61.48, H 4.77, N 11.21.

DPPH scavenging activity. DPPH scavenging activity of the compound **3** and its phthalocyanine complexes was determined spectrophotometrically by measuring the change in the absorbance of free radical (2,2-diphenyl-2-picrylhydrazyl) at 517 nm [20]. 0.5 mL DMF solutions of complexes were diluted with DMF to obtain solutions with different concentrations (5 ppm, 10 ppm, 25 ppm, 50 ppm, and 100 ppm). These samples were added to 2 mL of methanol solution of DPPH. The final mixtures were shaken vigorously and incubated in the dark for 60 min in an incubator at 25 °C. Absorbance values at 517 nm were measured using DMF solution as a blank control. Ascorbic acid and trolox were used as positive control. The scavenging effect was calculated using the following formula:

$$\text{scavenging activity (\%)} = (A_c - A_s) \div A_c \times 100,$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample. The data for antioxidant activities were presented as mean \pm SD of three determinations.

Metal chelating activity. The study of the metal chelating effects of compound **3** and its phthalocyanine complexes on ferrous ions was carried out as described in the literature [21]. One milliliter of DMF solution of the compound at different concentrations and 0.1 mL 2 mM $\text{FeCl}_2 \cdot \text{H}_2\text{O}$, 0.2 mL 5 mM ferrozine-1,2,4-triazine and 3.7 mL methanol were mixed in a test tube and allowed to react for 10 min. The absorbance of reactants was measured at 562 nm. The same mixture without compound was used as control. The minimum absorbance indicates the highest ferrous ion chelating capacity and is checked against EDTA as a positive control using formula given below:

$$\text{metal chelating effect: } (A_c - A_s) / A_c \times 100.$$

Reducing power. The compound **3** and its phthalocyanine complexes (5—100 mg/L) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] (1 %), and then the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of upper layer of the solution was mixed with 2.5 mL distilled water and 0.5 mL FeCl_3 (0.1 %), and the absorbance was measured at 700 nm [22]. α -Tocopherol was used as a positive control.

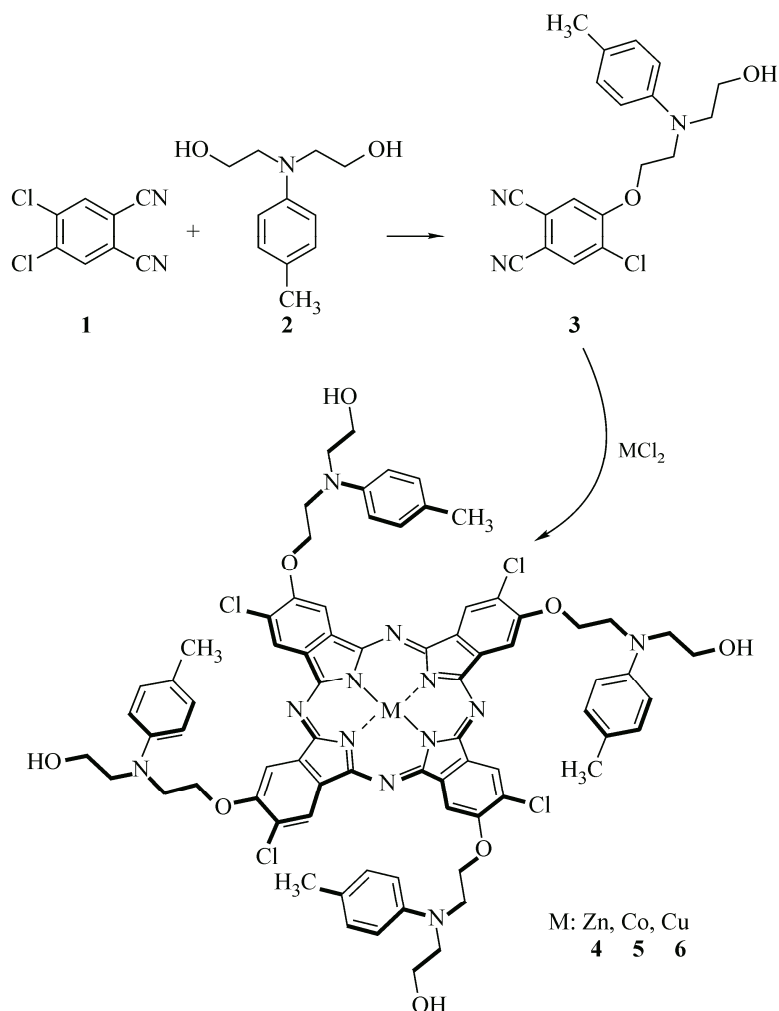
Antibacterial analyses. To determine the antimicrobial potential of the compound **3** and its phthalocyanine complexes, disc diffusion method was applied as described in the literature [23]. *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (6051), *Enterococcus hirae* (ATCC 10541), *Micrococcus luteus* (ATCC 9341), and *Pseudomonas aeruginosa* (ATCC 9027) were used as the test microorganisms. Nutrient broth (NB) was used for culturing of test bacteria. All strains were regenerated twice before use in the antimicrobial test.

RESULTS AND DISCUSSION

Synthesis and characterization. Phthalonitrile derivative **3** was obtained through the reaction of 2,2'-(4-methylphenylimino)diethanol **2** and 4,5-dichlorophthalonitrile **1** in dry DMF in the presence of dry K_2CO_3 . The phthalocyanine complexes **4—6** were obtained by heating the phthalonitrile **3** with ZnCl_2 , CoCl_2 , CuCl_2 metal salts under N_2 atmosphere. Scheme 1 shows the synthetic pathways for the compounds **3—6** used in this study. The characterization of the compounds was carried out by the combination of several methods, including elemental analysis, FT-IR, ^1H NMR, Mass spectrometry, and UV-Vis spectra.

The mass spectra of **3—5** confirmed the proposed structure exhibiting the expected peaks at $m/z = 355$ [M] $^+$, $m/z = 1488$ [M] $^+$, and $m/z = 1482.2$ [M] $^+$, respectively. The ^1H NMR spectrum of compound **3** was also in a good agreement with the structure of **3**. The ^1H NMR spectrum of **3** showed

aromatic ring protons between 8.34 ppm and 6.67 ppm as multiple peaks, OH proton at 3.45 ppm, aliphatic group protons at 4.38—3.78 ppm, and CH₃ protons at 2.50 ppm. The ¹H NMR spectrum of **4** exhibits the characteristic signals of aromatic protons at 8.98—6.64 ppm, OH proton at 3.47 ppm, aliphatic groups at 4.83—3.85 ppm, and CH₃ at 2.50—2.19 ppm.



Scheme 1. The route for the synthesis of compound **3—6**

The IR spectral data clearly indicated the formation of compound **3** by the appearance of new absorption bands at 3276 (OH), 3085—3015 (Ar—H), 2923 (CH₃), 2238 (CN), 1617—1575 (C=C), 1257 (Ar—O—CH). After conversion of the phthalonitrile derivative **3** into phthalocyanines **4—6**, the sharp peak for the (CN) vibrations disappeared [24]. The IR spectrum of **4** clearly indicated the formation of compound **4** by the appearance of new absorption bands at 3328 (OH), 3062 (Ar—H), 2910—2848 (CH₃), 1601—1517 (C=C), 1257 (Ar—O—CH). The IR spectra of **5—6** were very similar to the spectrum of **5** with slight shifts. The IR spectral data clearly indicated the formation of compounds **5—6** by the appearance of new absorption bands at 3330—3330 (OH), 3073—3067 (Ar—H), 2921—2919 (CH₃), 1605—1518 (C=C), 1257—1261 (Ar—O—CH), respectively, confirming the proposed structures.

Aggregation properties. The investigation of the UV-Vis spectra of phthalocyanines (**4—6**) confirmed the proposed structures. The aggregation behaviour of all these phthalocyanines have been measured in THF at a concentration range of 10^{-5} — 10^{-6} mol·L⁻¹ and results are presented in Fig. 1. These phthalocyanines are free from aggregation in this concentration range. The compounds showed

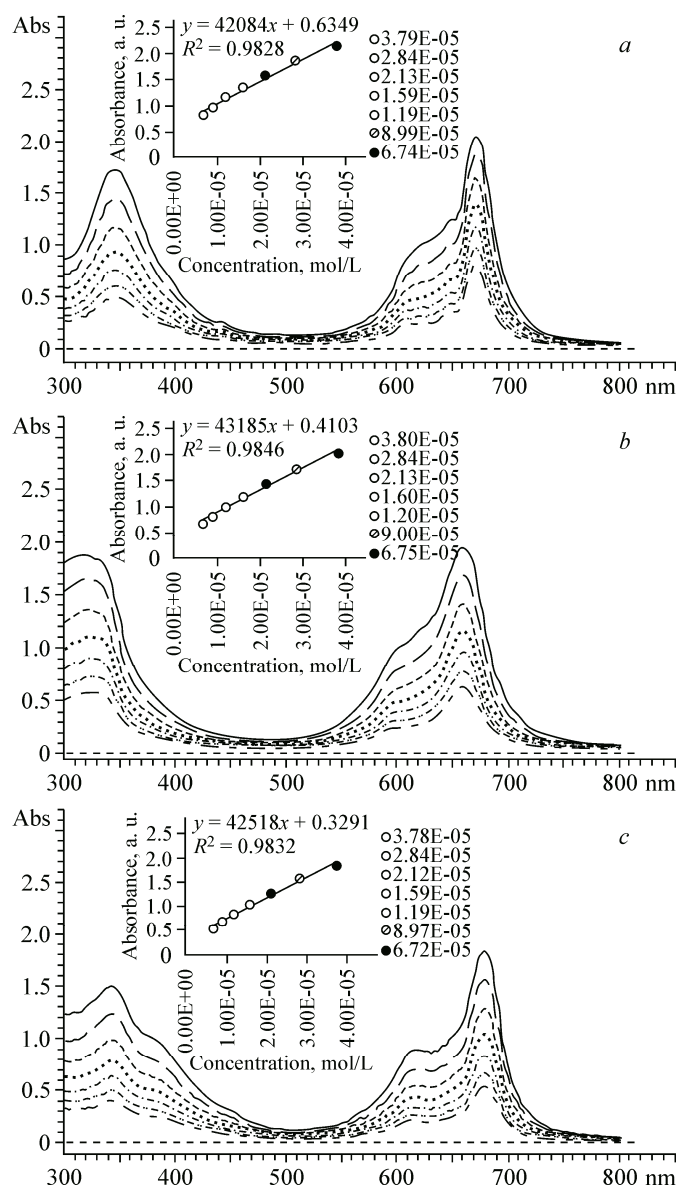


Fig. 1. The aggregation behaviour of phthalocyanine 4 (a), 5 (b), 6 (c)

characteristic absorptions in the *Q* band region at 670 nm for **4**, 660 nm for **5**, and 676 nm for **6**. Molecular aggregation is a common phenomenon in chemistry of PCS, particularly in water, and gives rise to broadening and blue-shift of their *Q* band when the chromophores are aligned in a face-to-face manner [25]. This aggregation-free characteristic is extremely important from the viewpoint of its application as photosensitizers in PDT because aggregation phenomena provide an efficient non radiative relaxation pathway and shorten lifetime of excited states in PCS, considerably reducing the photochemical activities of the macrocycles. Also, it is an intriguing subject from a viewpoint of supra-molecular chemistry considerably reducing the photochemical activities of the macrocycles [26].

DPPH scavenging activity. The antioxidant activity of the compound **3** and its phthalocyanine complexes were measured by means of their hydrogen donating or radical scavenging ability by UV-Vis spectrophotometer using the stable DPPH. In DPPH scavenging assay, the DMF solutions of the compound **3** and its phthalocyanine complexes showed weak activity when compared with standards (Table 1). The antioxidant activities of compounds increased with the increase in concentration (5 mg/L, 10 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L). Compound **4** exhibited the best activity by 35.2 % at 100 mg/L concentration. The results were found to be compatible with the literature values [27].

Table 1

Antioxidant activity of compounds and standards

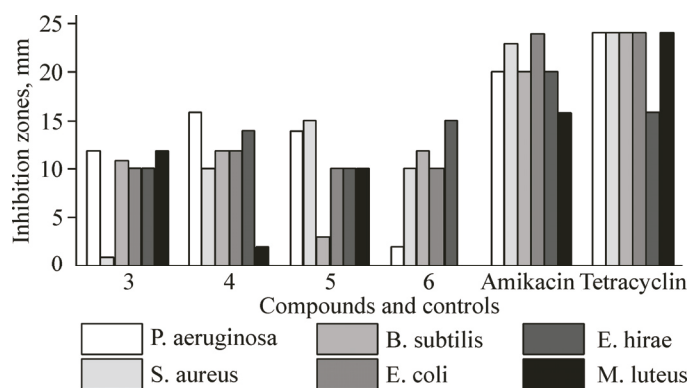
DPPH scavenging activity, %						
Concentrations	3	4	5	6	Ascorbic acid	Trolox
5 ppm	2.5	5.6	4.2	3.1	40.5	35.1
10 ppm	4.2	9.3	7.6	4.8	52.8	46.5
25 ppm	7.6	18.7	9.1	8.9	95.29	91.8
50 ppm	14.8	29.7	15.9	16.7	97.6	95.6
100 ppm	21.5	35.2	24.5	22.3	99.1	98.7

Metal chelating activity, %					
Concentrations	3	4	5	6	EDTA
5 ppm	13.2	8.7	5.2	7.6	90.3
10 ppm	19.5	19.8	10.3	13.4	95.3
25 ppm	33.4	32.3	16.4	19.7	97.6
50 ppm	48.2	41.6	21.6	24.9	100
100 ppm	69.7	56.4	30.2	35.8	100

Reducing power activity					
Concentrations	3	4	5	6	α -tocopherol
5 ppm	0.083	0.121	0.047	0.055	0.078
10 ppm	0.112	0.238	0.083	0.101	0.104
25 ppm	0.198	0.374	0.131	0.146	0.221
50 ppm	0.357	0.584	0.199	0.224	0.376
100 ppm	0.479	0.774	0.248	0.375	0.467

Metal chelating activity. Ferrozine can quantitatively form complexes with Fe^{2+} . However, by the means of chelating agents, the complex formation is prevented with the result that the red colour of the complex is diminished. With a coexisting chelator, spectrophotometric reduction of colour allows the estimation of the chelating activity. The transition metal ion, Fe^{2+} , possesses the ability to lose single electron by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [28]. Only two compounds (3 and 4) displayed above the average activity when compared with other compounds as can be seen in Table 1. EDTA showed precise activity even at low concentrations.

Reducing power activity. In this activity test, the yellow color of the test solution changes to green and blue depending on the reducing power capacity of each compound. The presence of reducers (i.e., antioxidants) trigger the reaction of the Fe^{3+} /ferricyanide complex to the ferrous form the Perl's Prussian blue after the addition of trichloroacetic acid and ferric chloride, that can be monitored at 700 nm. Compounds 3 and 4 exhibited higher activities than α -tocopherol which was used as a positive control. The activities were 0.479 for 3, 0.774 for 4 and 0.467 for α -tocopherol (Table 1).



The activities were 0.479 for 3, 0.774 for 4 and 0.467 for α -tocopherol (Table 1).

Antibacterial activity. Compounds 3—6 were tested for their antibacterial activity as seen at Fig. 2. The potential antibacterial activities of the compounds against *Escherichia coli* (ATCC 10536),

Fig. 2. Antimicrobial activity of compounds and controls

Staphylococcus aureus (ATCC 6538), *Bacillus subtilis* (6051), *Enterococcus hirae* (ATCC 10541), *Micrococcus luteus* (ATCC 9341), and *Pseudomonas aeruginosa* (ATCC 9027) were studied by disc diffusion method. Highest activity was obtained by compound **4** as 16 mm against to *P. aeruginosa*. The antibiotics that used for control Amicasin and Tetracyclin displayed excellent inhibition activities against all studied microorganisms [29]. These antibacterial activity results were similar to those reported in the literature.

CONCLUSIONS

The present work describes the synthesis, characterization and aggregation behavior of 4-chloro-5-(2-((2-hydroxyethyl)(*p*-tolyl)amino)ethoxy) substituted phthalocyanines and evaluation of their antioxidant and antimicrobial activities. The synthesized phthalocyanine complexes show excellent solubility in a wide range of organic solvents. These compounds do not show aggregation in the concentration range of 10^{-5} — 10^{-6} mol·L⁻¹ that makes them suitable candidates for PDT. In the light of the results obtained from the study, it can be said that compound **4** displayed higher activities than the other complexes for both antioxidant and antimicrobial activities. These activities may be further developed by some modifications on the compounds. The antioxidant activities of these compounds were found to be compatible with the literature values [16, 26].

This study was supported by the Research Fund of Yüzüncü Yıl University (2013-FBE-D059).

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