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SYNTHESIS, CHARACTERIZATION, AGGREGATION BEHAVIOR, ANTIOXIDANT ACTIVITY, AND ANTIBACTERIAL ACTIVITY OF METALLOPHTHALOCYANINES CARRYING FOUR PHTHALONITRILES GROUP

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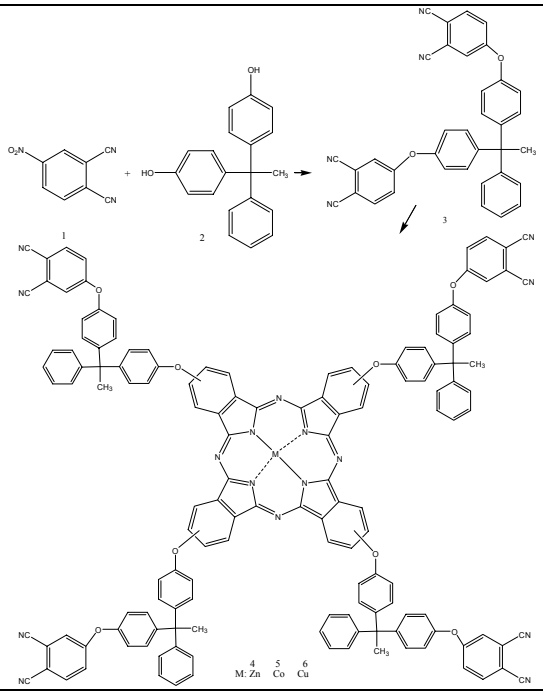
A novel phthalonitrile derivative and zinc (II), cobalt (II), and cupper (II) phthalocyanines complexes were synthesis and characterized. The novel compounds have been characterized by using various spectroscopic data FTIR, UV/vis, NMR, elemental analysis etc. The aggregation investigations carried out in different concentrations indicate that 4- (4-(1-(4-phenoxyphenyl)-1-phenylethyl)phenoxy)phthalonitrile substituted phthalocyanine compounds do not have any aggregation behavior for the concentration range of 10^{-4}-10^{-5} M in tetrahydrofuran. The antioxidant properties of the phthalonitrile and its phthalocyanine compounds were evaluated in three series of in vitro tests: DPPH free radical scavenging, ferrous ion chelating activity and reducing power. Antimicrobial activities of compounds were investigated.



INTRODUCTION

Phthalocyanines, have been utilized in important functional materials, such as light emitting devices,[1] chemical sensors,[2,3] optical data storage,[4,5] solar cells ,[6,7] catalysis etc.[8] One of their most important applications is as photosensitizers in photodynamic therapy (PDT).[9-11] The aggregation for applications photodynamic therapy is disadvantage.[12] Phthalocyanines in water have a strong tendency for stacking (or aggregate) to form dimers and high order oligomers, induced by the tendency of the hydrophobic skeleton

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to avoid the contact with water.[13] Stacking decreases photosensitizing efficacy because of energy transfer between the aggregated molecules. The application for PDT calls for specific phthalocyanine structures with distinct physical and chemical properties hence immense research has gone into the preparation and incorporation of substituents that improve such properties.[14,15] One of which includes the synthesis of phthalocyanine derivatives with different substituents on the ring. [16,17]

In this study, we have described the synthesis and characterization of phthalocyanines bearing 4- (4-(1-(4-phenoxyphenyl)-1-phenylethyl)phenoxy) phthalonitrile substituents on the peripheral positions. We have also reported the results of a comprehensive investigation of the concentration effects on the aggregation properties of phthalocyanine derivatives in THF. The antioxidant and antimicrobial activities of these complexes are also investigated in this work as potential photosensitizers for PDT.

EXPERIMENTAL

General Remarks

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), ferrous chloride, 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)- 1,2,4-triazine (Ferrozine), ascorbic Acid, trolox, α-tocopherol and dimethylformamide (DMF) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Blank antimicrobial susceptibity test discs were obtained from Oxoid. $ZnCl_2$, $CoCl_2$, $CuCl_2$, K_2CO_3, $CHCl_3$, THF, DMSO and DBU were purchased from Merck and Sigma. The solvents were purified according to standard procedure[18] and stored over molecular sieves (4 Å). All reactions were carried out under dry nitrogen atmosphere**.** 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). ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer (Malatya IBTAM, Central Laboratory, Turkey) with tetramethylsilane as internal standard.

Synthesis

4-4'-(((1-phenylethane-1,1-diyl)bis(4,1-phenylene)bis(oxy) diphthalonitrile **(3)**

A mixture of 4-(1-(4-hydroxyphenyl)-1-phenylethyl) phenol 2 (1.44g, 5 mmol) and 4-nitrophthalonitrile 1 (1.73 g, 10 mmol) in 25 mL dimethylformamide (DMF) was stirred at room temperature under nitrogen atmosphere. After stirring for 15 min, K_2CO_3 (2.2 g, 48 mmol) was added into the mixture over a period of 2 h. After stirring the reaction mixture for a further 24 h, the reaction mixture was poured into water (150 mL) and stirred. The precipitate was filtered, washed with water, and dried in vacuum. The product is soluble in THF, ethanol, acetone, ethyl acetate, CH_2Cl_2, $CHCl_3$, DMF and DMSO. Yield; 2.50 g(92 %). Mp: 120ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 8.10-7.11(Ar-H), 2.17(CH_3). FTIR υ_{max}/cm^{-1}: 3075(Ar-CH), 2981(CH_3), 2232(CN), 1591, 1249, 1174, 1115, 838, and 703. Anal calculated for $C_{36}H_{22}N_4O_2$: C 79.69; H 4.09; N 10.33 %. Found C 79.56; H 4.64; N 10.18 %.

2,10,16,24–[tetrakis 4-(4- (1- (4-phenoxyphenyl) -1-phenylethyl) phenoxy) phthalonitrile) phthalocyaninato] zinc (II) **(4)**

A mixture of 4-4'-(((1-phenylethane-1,1-diyl) bis (4,1-phenylene) bis (oxy) diphthalonitrile **3** (0.100 g, 0.184 mmol) and $ZnCl_2$ (0.017 g) was powdered in a quartz crucible and heated in a sealed glass tube for 5 min under nitrogen at 220-230 ºC. After cooling to room temperature, the product was washed with cold and hot ethanol, methanol several times and was filtered. Then, of soluble in THF was taken and dried. The product is soluble in DMF, THF and DMSO. The yield was 0.078g (76 %). Calc. for $C_{144}H_{88}N_{16}O_8Zn$: C, 77.36; H, 3.97; N, 10.02 %. Found: C, 77.25; H, 4.00; N, 9.99 %. ^{1}H NMR (DMSO-d_6) δ ppm: 8.11-6.45(A-Hr); 2.18(CH_3). UV-Vis (THF) λmax(log □: 674(5.13), 608(4.48). IR spectrum (cm^{-1}): 3057(Ar-CH), 2977(CH_3), 2231(CN) 1593, 1236, 1236, 1171, 1118, 838, and 701.

2,10,16,24–[tetrakis4-(4-(1-(4-phenoxyphenyl)-1-phenylethyl) phenoxy)phthalonitrile) phthalocyaninato] cobalt (II) **(5)**

The procedure is similar to the synthesis of **4** from **3**, using $CoCl_2$. The product is soluble in DMF, THF, and DMSO. The yield was 0.068 g (66 %).Calc. for $C_{144}H_{88}CoN_{16}O_8$: C, 77.58; H, 3.98; N, 10.05 %. Found: C, 77.63; H, 3.95; N, 10.01 %. UV-Vis ($CHCl_3$) λmax(log □): 666(5.26). IR spectrum (cm^{-1}): 3057(Ar-CH), 2974(CH_3), 2231(CN), 1591, 1243, 1170, 1117, 1091, 1055, 837, and 701.

2,10,16,24–[tetrakis 4-(4-(1-(4-phenoxyphenyl)-1-phenylethyl) phenoxy) phthalonitrile) phthalocyaninato] copper (II) **(6)**

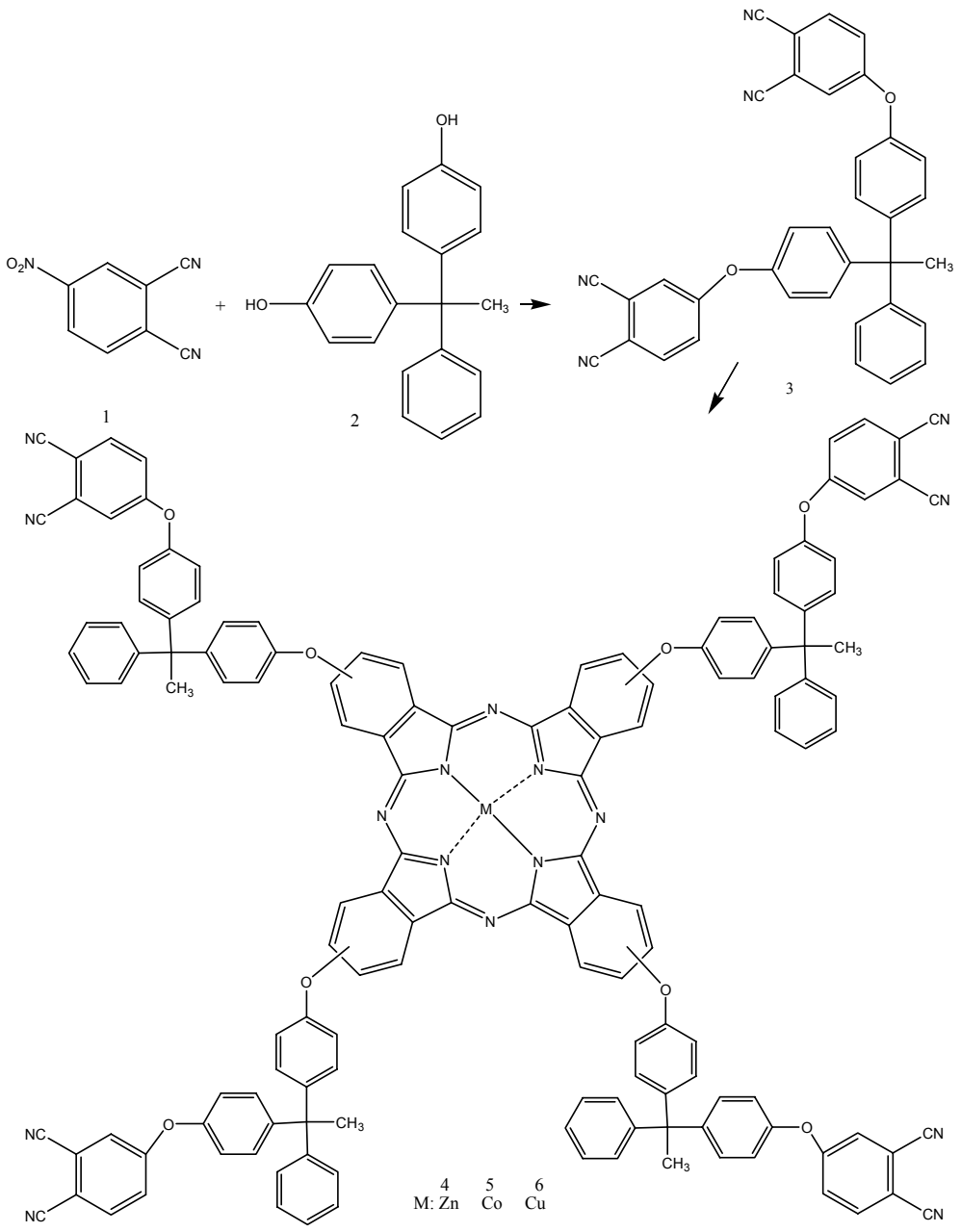
The procedure is similar to the synthesis of **4** from **3**, using $CuCl_2$. But the mixture is heated in a sealed glass tube for 5 min under nitrogen at 220 ºC. The product is soluble in DMF, THF, and DMSO. The yield was 0.086 g (83%). Calc. for $C_{112}H_{80}CuN_8O_8$: C, 77.42; H, 3.97; N, 10.03 %. Found: C, 77.39; H, 3.93; N, 9.98 %. UV-Vis (THF) λmax(log □): 674(5.31), 608(4.88). IR spectrum (cm^{-1}): 3073(Ar-CH), 2978(CH_3), 2231 (CN), 1589, 1247, 1173, 1114, 1087, 1014, 836, and 702.

DPPH radical scavenging activity

The compounds DMF stock solution was diluted to final concentration of 5-100 µg/mL with methanol. The methanol solution of 1.6 mL DPPH was added to test compounds (400µL) at different concentrations. The mixture was shaken vigorously and incubated at room temperature in dark for 30 min at room temperature. After the incubation time, the absorbance of the solution was measured at 517 nm by using UV-vis spectrophotometer. The DPPH radical scavenger effect was calculated using the following equation:

$$\text{Scavenging activity (\%}_{\text{control}}) = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate. Ascorbic acid and trolox were used as positive control.



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

Chelating ability on ferrous ions

The chelating activity of the phthalonitrile and its metallophthalocyanine complexes toward Fe^{2+} was determined by the method of Dinis.[19] Different concentration of methanol solution of the test compounds was mixed with 50 µL 2mM $FeCl_2$ and 1.85 mL of methanol then was added. The reaction was initiated with addition of 100 µL of 5 mM ferrozine. After 10 min incubation at room temperature, the absorbance of test compound was measured at 562 nm using UV-vis spectrophotometer. EDTA was used as a control. The result of chelating activity of the test samples was calculated by using the formula given;

$$\text{Chelating activity (\%)} = (A_{control} - A_{sample})/A_{control} \times 100;$$

where $A_{control}$ is the absorbance of the control reaction, and A_{sample} represents the absorbance obtained in the presence of compounds or EDTA, the percentage of inhibition of the ferrozine–Fe^{2+} complex formation was calculated.

Determination of reducing power

The reducing power of test compounds was experimented by using the method of Oyaizu.[20] Different concentrations of test compounds (1.25 mL, 5-100 mg/L) were added to 1.25 mL of 200 mM sodium phosphate buffer (pH 6.6) and 1.25 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After 20 min, 1.25 mL of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 1000 rpm for 8 min. Five milliliter of the supernatant solution was mixed with 1.25 mL of deionised water and 0.25 mL of 0.1% of ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. α-tocopherol were used as standards.

Antibacterial activity

The phthalonitrile, its metallo-phthalocyanine complexes and control (DMSO) were tested for in vitro antibacterial activity against *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus cereus*, *Bacillus subtilis* (6051), *Legionella pneumophila* subsp *pneumophiia* (ATCC 33152), *Micrococcus luteus* (ATCC 9341) and *Enterococcus hirae* (ATCC 10541). Sterile blank antibacterial test discs (6 mm diameter, Oxoid) were soaked with 15μL of the phthalonitrile, its metallo-phthalocyanine complexes solution in methanol, dried, and then placed on nutrient agar plates. After 24 hour incubation at 37°C, antibacterial activities were evaluated by measuring the inhibition zone diameters in mm around each disc. The results were compared with Amikacin (30 μg), Erythromycin (15 μg), Vancomycin (30 μg) which obtained from Oxoid.

RESULTS AND DISCUSSİON

Synthesis and characterization

The 4-4'-(((1-phenylethane-1,1-diyl)bis(4,1-phenylene)bis(oxy)diphthalonitrile **3** was obtained through the reaction between 4-(1-(4-hydroxyphenyl)-1-phenylethyl)phenol **2** and 4-nitrophthalonitrile **1** in dry DMF in the presence of dry K_2CO_3. The complexes **4-6** were synthesized by the reaction of **3** with $ZnCl_2$, $CoCl_2$, $CuCl_2$ under N_2 atmosphere. The route for the synthesis of compounds **3-6** is shown in Scheme 1. The characterization of the compounds was carried out by the combination of several methods, including elemental analysis, IR, ^{1}H NMR, and UV–vis spectra. The compounds structures were confirmed by the results of these analyses.

The structures of compounds **3-4** are confirmed by ^{1}H NMR for both the phthalonitrile derivative and phthalocyanine through aromatic ring protons in their respective regions. In the ^{1}H NMR spectrum of compound **3** in DMSO-d_6, aromatic protons appear at 8.10-7.11(Ar-H), and aliphatic protons at 2.17(CH_3) ppm. In the ^{1}H NMR spectrum of compound **4** within DMSO-d_6, aromatic protons appear at 8.11-6.45(A-Hr), aliphatic protons at 2.18(CH_3) ppm. ^{1}H NMR measurement of compound **5-6** were excluded due to their paramagnetic properties.

Comparison of the IR spectral data clearly indicated the formation of compound **3** by the appearance of new absorption bands at 3075(Ar-CH), 2981(CH_3), 2232(CN), 1591(C=C), 1249(Ar-O-Ar). Phthalocyanines **4–6** possessed similar IR spectra with small shift. The IR spectra of compounds **4-6** displayed Ar-O-Ar peaks at 1236-1247 cm^{-1}, C=C (in phenyl rings) peaks at 1589-1593 cm^{-1}, aromatic CH peaks at 3057-3073 cm^{-1}, aliphatic CH peaks at 2974-2978 cm^{-1}.

The UV-vis spectra of the phthalocyanine **4–6** in THF showed characteristic Q band absorptions between 674–666 nm, which were attributed to the π-π* transition from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) of the Pcs ring. The other bands (B) in UV region were observed due to transition from the deeper π levels to the LUMO. The spectra showed monomeric behavior evidenced by a single Q band, typical of metallophthalocyanines compounds for **4-6** in THF, which depict the monomeric nature of the these complexes in this solvent.

Aggregation properties

Generally in phthalocyanines increasing the concentration leads to aggregation, which is easily observed by the values of the Q bands, which shift to higher energies by a parallel decrease in molar absorption coefficient. Aggregation is usually depicted as a coplanar association of rings progressing from monomer to dimer and higher order complex.[21-22] It is dependent on the concentration, nature of the solvent, nature of the substituents, complexed metal ions and temperature. Non-aggregated phthalocyanines have received considerable attention. These compounds, normally with bulky substituents, possess good solubility, which can facilitate the purification and characterization processes. The non-aggregated nature can also prevent undesirable effects arising from stacking of molecules.[23] In the present work, the aggregation behavior of the metallophthalocyanine complexes **5** and **6** were also investigated at different concentrations in THF (Fig. 1 for compound **5** and Fig. 2 for compound 6). In THF, as the concentration was increased, the intensity of absorption of the Q band corresponding to monomeric species also increased and there were no new bands due to the aggregated species for both of the complexes. Beer-Lambert law was obeyed in the concentrations ranging from 2.5×10^{-5} to 1×10^{-4} for compound **5** and from 7.39×10^{-5} to 3.11×10^{-4} M for compound **6**, respectively. Due to the nature of 4- (4-(1-(4-phenoxyphenyl)-1-phenylethyl)phenoxy)phthalonitrile substituents, it can clearly be concluded that the phthalocyanines derivatives (**5** and **6**) did not show aggregation in THF for the studied concentrations.

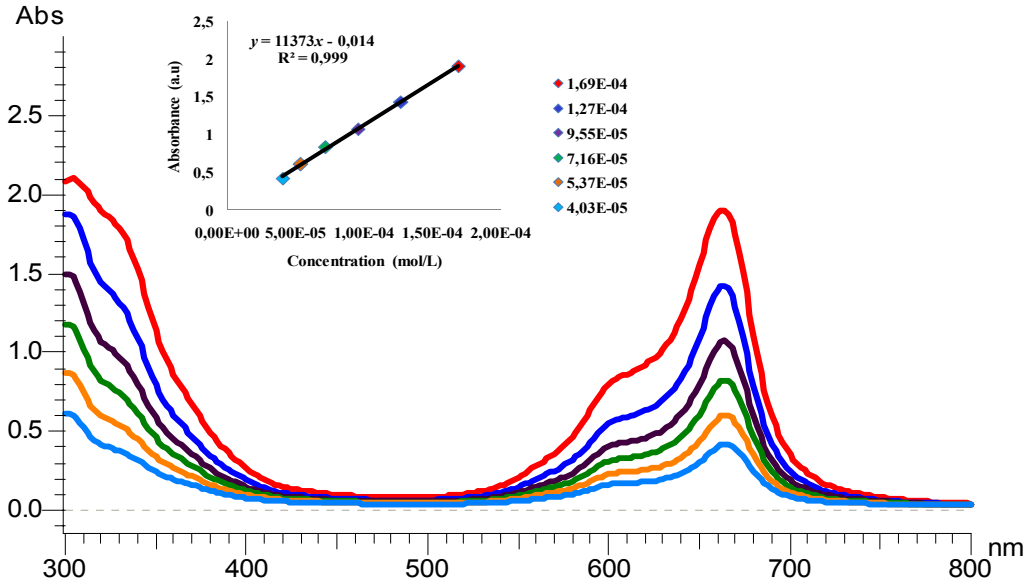


Fig. 1 – The aggregation behaviour of phthalocyanine **5**.

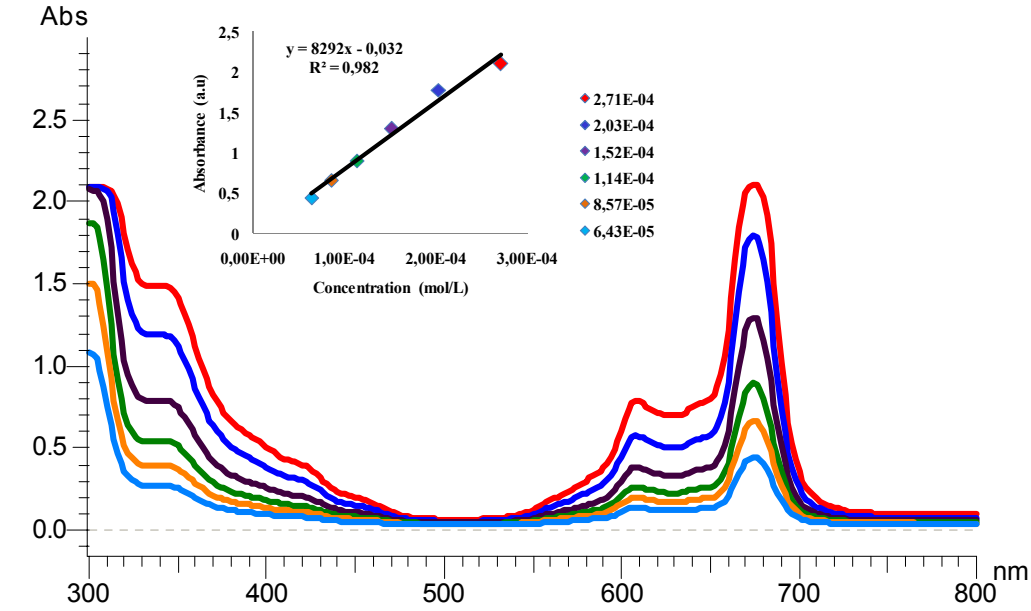


Fig. 2 – The aggregation behaviour of phthalocyanine **6**.

DPPH radical-scavenging activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities of organic and inorganic compounds in a relatively short time.[24] The effect of antioxidants on DPPH radical scavenging is due to their hydrogen donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.[25] Fig. 3 illustrates the DPPH free radical scavenging ability of the phthalonitrile, its metallo-phthalocyanine complexes and standards. DPPH scavenging activity of compounds also increased with increasing concentration. The scavenging ability of the phthalonitrile, its metallo-phthalocyanine complexes at 250 mg/L on the DPPH radical scavenging were in the following order of **6** > **5** > **3** > **4** and were found to be 37.21%, 16.05%, 10.57 and 9.66%, respectively at the same concentration. Ascorbic asid and trolox were showed higher DPPH scavenging activity than the phthalonitrile, its metallo-phthalocyanine complexes at all concentration. The results of DPPH scavenging activities of tested compounds were showed also good agreement with literature [24] and prior reports. [26]

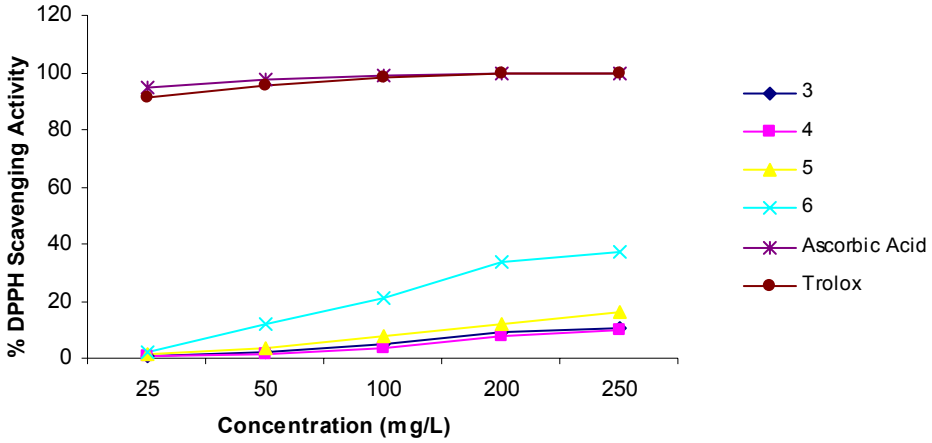


Fig. 3 – Radical-scavenging activity on DPPH radicals (%) of the compounds and standards.

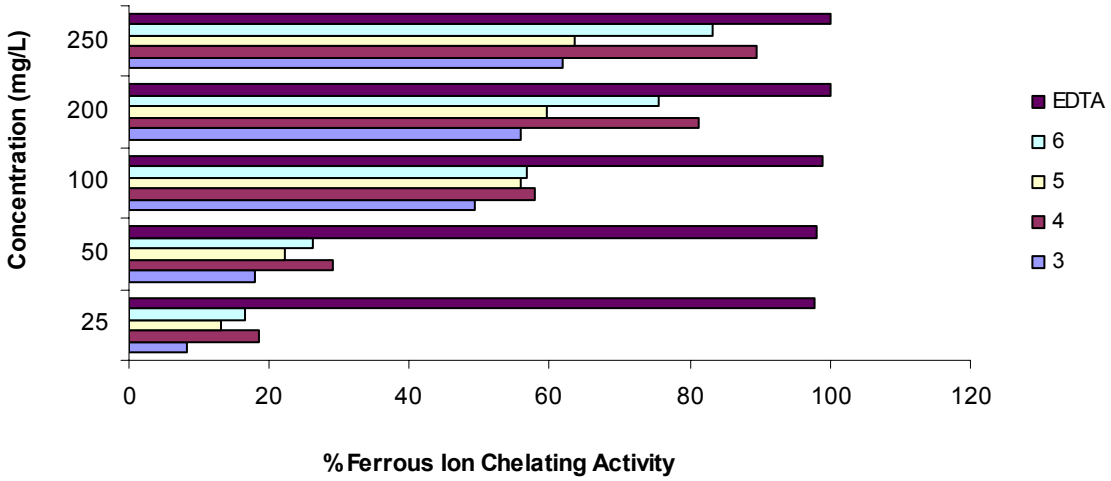


Fig. 4 – Chelating effect of compounds and EDTA on ferrous ion.

Metal chelating activity on ferrous ions

The transition metal ion, Fe^{2+} possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals.[27] The chelating effects of the compounds were compared with EDTA as standard. As can be seen from Fig. 4, the metal chelating activity of the phthalonitrile, its metallophthalocyanine complexes increased with the increasing concentration. The chelating activity of samples were lower than EDTA at all concentration. The chelating activity of the test samples and EDTA follow the order: EDTA >**4** >**6** >**5** >**3** at 200 mg/L. All tested compounds interfered with the formation of ferrous and ferrozine complex, indicating that they have chelating activity. The maximum ferrous chelating activity was obtained with **4** at a concentration of 250 mg/L (89.49%), while **3** showed the minimum chelating activity at concentration of 25 mg/mL (8.29%). Dundar reported [28] the metal chelating activity as 62.5% for the methanolic extracts of *Pleurotus ostreatus*. The chelating activity of **4** and **6** were showed higher than methanolic extracts of *P. ostreatus*.

Reducing power

In this study, the color of the test the phthalonitrile, its metallo-phthalocyanine complexes solutions changed from yellow to the various color of green and blue depending upon their reducing power of these antioxidants (Fig.5). [29] The presence of antioxidant substance induces the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. The results showed that the maximum reducing power of **3**, **4**, **5**, **6** and α-tocopherol were 0.389, 0.112, 0.153, 0.136 and 0.603, respectively at concentration of 250 mg/L. The results of **4** and **5** were showed also in good agreement with the previous reports [30] and while **3** and **6** were demonstrated higher reducing activity.

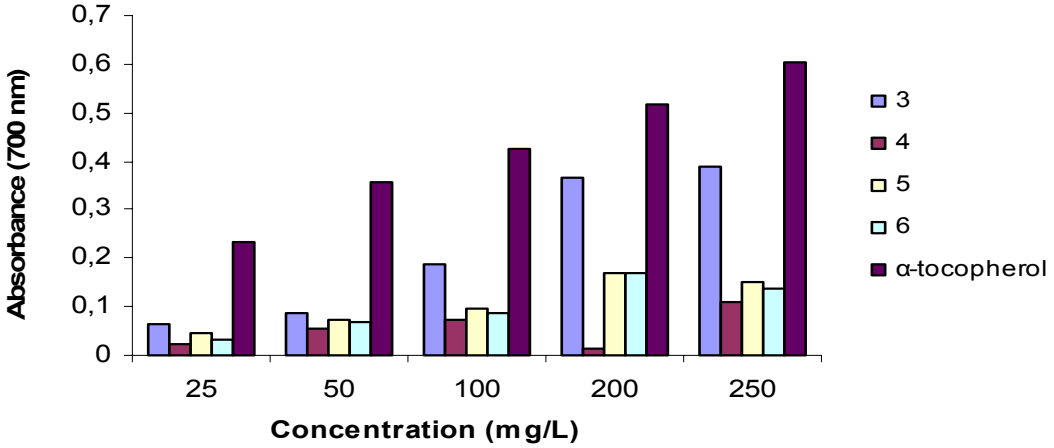


Fig. 5 – Reducing power of compounds and α-tocopherol.

Table 1

The antibacterial activity of compound **3-6**

	Compounds and standart antibiotic discs[a]						
Bacteria	**3**	**4**	**5**	**6**	**AK**	**VA**	**E**
P. aeriginosa	8	9	9	7	20	7	16
B.cereus	7	8	8	8	16	12	20
L. pneumophila subsp pneumophiia	9	7	9	9	16	12	16
B. subtilis	0	10	7	8	20	16	16
M. luteus	9	8	13	14	16	16	24
E. hirae	8	9	10	11	20	10	10

[a] Inhibition diameter in millimeters. Inhibition diameter. AK= Amikacin (30 μg), E15= Erythromycin (15 μg), Vancomycin (30 μg).

Antibacterial activity

The antibacterial activities of the phthalonitrile and its metallophthalocyanine complexes were studied for their effect on certain bacteria such as *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus cereus*, *Bacillus subtilis* (6051), *Legionella pneumophila* subsp *pneumophiia* (ATCC 33152), *Micrococcus luteus* (ATCC 9341) and *Enterococcus hirae* (ATCC 10541). Results are presented in Table 1. **3**, **4** and **5** showed more antibacterial activity than Vancomycin (30 μg) against *P. aeruginosa* (ATCC 9027). The results of antibacterial activity study, revealed that all tested samples had a weak antibacterial activity against *B. cereus* and **3** actually no antibacterial activity against *B. subtilis* (6051). Amikacin and Erythromycin exhibited more antibacterial activities than test samples against tested bacteria. Antibacterial activities were in the order of **6** > **5** > **3** > **4** for *M. luteus*, **6** > **5** > **4** > **3** for *E. hirae*, **5** = **4** > **3** > **6** for *P. aeriginosa*. The strongest antibacterial activity was achieved with **6** against *M. luteus*.

CONCLUSIONS

The present work describes the synthesis and characterization of phthalocyanines bearing 4-(4-(1-(4-phenoxyphenyl)-1-phenylethyl)phenoxy)phthalonitrile substituents on the peripheral positions. The synthesized phthalocyanine complexes show solubility in organic solvents such as, THF, DMF and DMSO. The aggregation behaviors of compound **5** and **6** were investigated. These phthalocyanines showed monomeric behaviors in THF for studied concentration ranges. 4- (4-(1-(4-phenoxyphenyl)-1-phenylethyl)phenoxy)phthalonitrile groups can be efficient substituents to keep phthalocyanine macrocycles away from aggregation. In addition, the antioxidant activities of compounds were investigated. The antimicrobial activity results showed that most of the synthesized complexes possessed moderate antibacterial activity against tested bacteria. The antioxidant activity of the samples for DPPH, metal chelating and reducing power were concentration dependent.

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