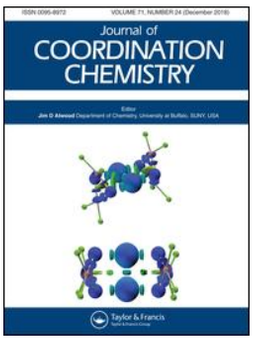

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Octa- and tetra-substituted phthalocyanines with methoxyeugenol group: synthesis, characterization and *in vitro* antimicrobial activity

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ABSTRACT

In this study, the novel phthalonitrile derivatives **1** and **2** were synthesized. Tetra-substituted metal-free **1a** (H_2Pc) and metallophthalocyanines (ZnPc **1b**, CoPc **1c** and CuPc **1d**) bearing four peripheral 4-allyl-2,6-dimethoxyphenol (methoxy-eugenol) groups and octa-substituted metal-free **2a** and metallophthalocyanines (ZnPc **2b**, CoPc **2c** and CuPc **2d**) bearing eight peripheral methoxy-eugenol group were synthesized. All new synthesized compounds are characterized by IR, $^{13}C\{^1H\}$ NMR (for **1** and **2**), UV-Vis (for **1a**–**2d**) and mass spectroscopy. Antimicrobial activities of the synthesized phthalonitrile derivatives (**1** and **2**) and phthalocyanine compounds **1a**–**2d** were determined by microdilution broth method against nine different bacteria and one yeast species. In this study, CoPc (**2c**) compound, which has eight peripheral 4-allyl-2,6-dimethoxyphenol, had the highest antibacterial activity (minimum inhibitory concentration [MIC] = 312 μg/mL). In addition, **1b**, **1d**, **2b** and **2d** were found to be effective on at least three different bacterial species at different concentrations (MIC = 1250–5000 μg/mL). *Shigella flexneri* were the most affected bacteria, but none of the compounds were effective against fungal isolate.

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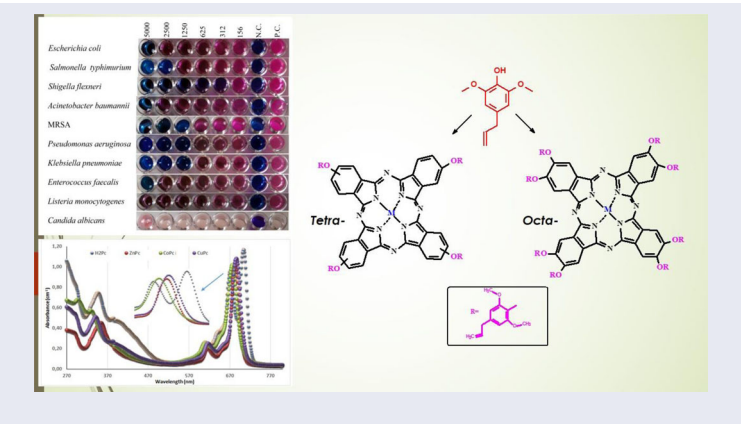


1. Introduction

Phthalocyanine compounds are important macrocyclic compounds and they form one of the analogues of porphyrin [1, 2]. These compounds are used in many applications such as photodynamic therapy, electrochemistry, catalytic and photocatalytic, solar cells, anticancer and antimicrobial [3–9]. Their low solubility in prevalent organic solvents induces difficulties for many applications. On account of this reason, one of the significant aims of research on the chemistry of phthalocyanines is to augment their solubility in diverse solvents [10]. The solubility of MPc compounds can be enhanced by the incorporation of substituents, such as phenoxy, alkyl, alkoxy and bulky or long-chain groups at peripheral and non-peripheral position to the Pc core [11–13]. Eugenol group substituted phthalocyanine compounds can enhance their solubility [14–16]. The number of connected substituents and the difference of metal atom in the center distinguish the molecules of the phthalocyanine to be synthesized.

Eugenol is an important phenolic compound and it is found in natural products such as clove, clove oil, coconut, cinnamon, basil and bay leaf [17]. This natural compound has diverse biological features such as antioxidant, local anesthetic, anti-inflammatory, antimicrobial, analgesic, antimutagenic and anticarcinogenic effect [18, 19].

Antibiotics used in the treatment of bacterial infections have been used extensively for many years since the beginning of the 20th century. Unconscious and overuse of antibiotics that are bacteriocidal or bacteriostatic effective may cause resistance development and result in the spread of resistant pathogens. Approximately 20 million patients die annually due to bacterial infections, while new drug development studies are ongoing to combat resistant pathogens. Due to the development of technology, a variety of new agents that are effective against bacteria have been synthesized and used for the treatment of different bacterial infections [20, 21].

Significant progress has been made in the research of phthalocyanine derivatives as antioxidant and antibacterial activities in recent years [22]. To the best of our knowledge, there are a few studies on the antioxidant properties of eugenol-substituted phthalocyanine complexes in the literature [16, 23–25]. However, there are no antimicrobial studies of eugenol-substituted phthalocyanine compounds. In this study, in

order to improve the novelty of the study in the literature, the synthesis, spectral and antimicrobial features of metal-free (**1a**), zinc (**1b**), cobalt (**1c**) and copper (**1d**) phthalocyanine complexes bearing four 4-allyl-2,6-dimethoxyphenol groups at the peripheral positions and peripheral octa-substituted metal-free (**2a**), zinc (**2b**), cobalt (**2c**) and copper (**2d**) phthalocyanine complexes were studied (Scheme 2). We investigated the antibacterial and antifungal activities of the newly synthesized compounds by microdilution broth method and determined the minimum inhibitory concentration (MIC).

2. Experimental

2.1. Materials

The used materials and equipments were supplied as supplementary information.

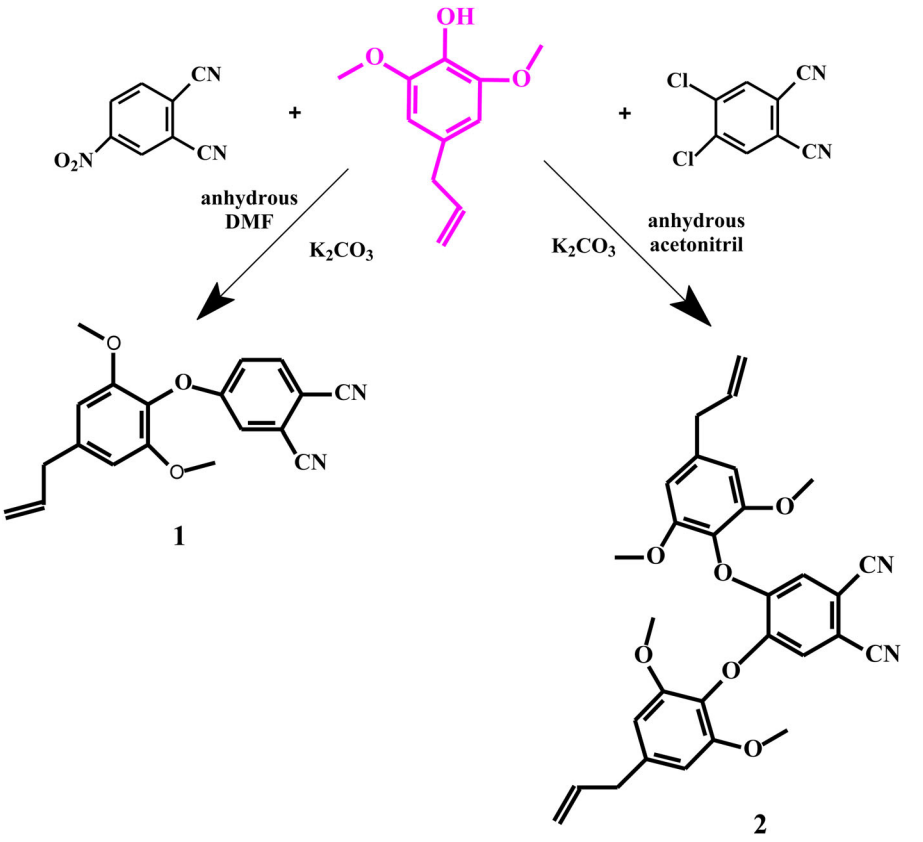
2.2. Synthesis

2.2.1. Synthesis of 4-(4-allyl-2,6-dimethoxyphenoxy)phthalonitrile (1)

4-Nitrophthalonitrile (0.89 g, 5.15 mmol) and 4-allyl-2,6-dimethoxyphenol (1 g, 5.15 mmol) were dissolved in dry DMF (15 mL) and the solution was stirred at 55 °C. Then, dry K_2CO_3 (2.13 g, 15.45 mmol) was added into this solution the portion wise during 2 h. The system was stirred under N_2 at same temperature for 135 h. After this time, the reaction mixture was poured into ice-water and stirred at room temperature. The mixture was filtered, dried in vacuum and recrystallized from ethanol to orange powder product (Scheme 1). Yield: 1.4 g (85%), m.p.: 145–149 °C. IR (ATR), ν_{max}/cm^{-1}: 3083 $(C\text{-}H)_{Ar}$, 3011–2840 $(C-H)_{Aliph}$, 2229 (C≡N), 1601–1588 (C=C), 1505, 1465, 1484, 1341, 1275, 1246, 1127, 819, 858, 712. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.71–7.68 (d, 1H, Ar–H), 7.28–7.16 (m, 2H, Ar–H), 6.52 (s, 2H, Ar–H), 6.02–5.98 (m, 1H, =C–H), 5.21–5.16 (m, 2H, $=CH\text{–}CH_2$), 3.78 (s, 6H, $O\text{–}CH_3$), 3.44–3.42 (d, 2H, $–CH_2$). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 161.86, 152.36, 139.64, 136.49, 135.07, 128.14, 120.27, 119.93, 117.25, 116.75, 115.70 (C≡N), 115.34 (C≡N), 108.13, 105.38, 56.08 ($O\text{–}CH_3$), 40.68 ($C\text{–}CH_2$). MS (m/z): Calculated: 320.35; Found: 321.24 $[M + H]^+$.

2.2.2. Synthesis of 4,5-bis(4-allyl-2,6-dimethoxyphenoxy)phthalonitrile (2)

4-Allyl-2,6-dimethoxyphenol (1 g, 5.15 mmol) was dissolved in 20 mL dry acetonitrile under nitrogen and anhydrous K_2CO_3 (2.13 g 15.45 mmol) was added at 50 °C. After stirring for 30 min at 50 °C, when the temperature was increased to 90 °C, 4,5-dichlorophthalonitrile (0.51 g 2.57 mmol) was added dropwise for 1 h and the reaction mixture was stirred at 90 °C for 166 h. Then, the mixture was filtered off with black filter paper and organic phase was removed under vacuum. The resulting crude product was dissolved in 15 mL of chloroform ($CHCl_3$). The mixture was washed with 15 mL of water. The organic phase was dried over $MgSO_4$, filtered and rotary evaporated. The obtained oily brown product was purified by crystallization from ethyl alcohol to cream colored product (Scheme 1). Yield: 0.48 g (36%), m.p.: 147–150 °C. IR (ATR), ν_{max}/cm^{-1}: 3079 $(C\text{-}H)_{Ar}$, 3032–2842 $(C-H)_{Aliph}$, 2233 (C≡N), 1599–1502 (C=C), 1464, 1418, 1336, 1293, 1279, 1209, 1126, 916, 822, 701. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.84 (s, 1H, Ar–H),

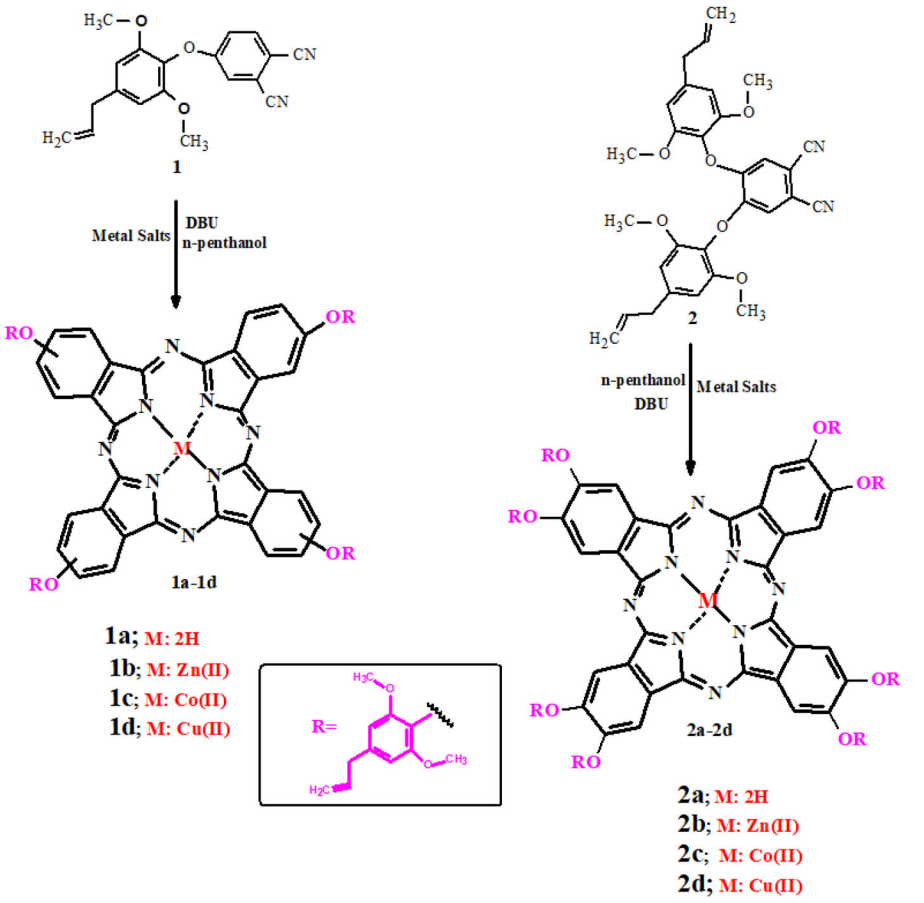


Scheme 1. The synthesis route of phthalonitrile derivatives **1** and **2**.

7.28 (s, 2H, Ar–H and $CDCl_3$), 6.87 (s, 2H, Ar–H), 6.53 (s, 4H, Ar–H), 6.07–5.98 (m, 2H, =CH), 5.21–5.17 (m, 4H, $=CH_2$), 3.90–3.80 (m, 12H, O–CH_3), 3.45–3.43 (d, 4H, C–CH_2). ^{13}C NMR (400 MHz, $CDCl_3$), δ (ppm): 152.49, 152.14, 136.62, 136.41, 135.12, 118.84, 118.63, 116.81, 116.63 (C≡N), 105.65, 105.51, 56.23 (O–CH_3), 40.67 (C–CH_2). MS (m/z): Calculated: 512.56; Found: 513.20 $[M + H]^+$.

2.2.3. Tetra-peripheral eugenol-substituted phthalocyanines (1a–1d)

Phthalonitrile derivative (**1**) (0.1 g, 0.31 mmol) and DBU (1.8-diazabicyclo[5.4.0]undec-7-ene) (10 drop) in 3 mL of dry pentanol was heated and stirred at 160 °C in a sealed glass tube for 24 h under N_2. After cooling to room temperature the green crude product was precipitated with ethanol, filtered, washed with ethanol and then dried *in vacuo*. Finally, pure metal-free phthalocyanine (**1a**) was obtained by column chromatography using silicon oxide and $CHCl_3$:CH_3OH as solvent system. Substituted phthalonitrile (**1**) (0.1 g) and anhydrous metal salts ($Zn(AcO)_2$ (29 mg, 0.56 mmol) for **1b**; $CoCl_2$ (20 mg, 0.56 mmol) for **1c**; $CuCl_2$ (21 mg, 0.56 mmol) for **1d**) were dissolved in 3 mL of n-pentanol and 10 drops DBU. Mixture temperature was raised to 160 °C and stirred for 24 h under nitrogen. After this time, ethanol was added to the medium and the green raw products were filtrated. Synthesized metallophthalocyanine complexes were



Scheme 2. The synthesis route of phthalocyanine compounds **1a**–**1d** and **2a**–**2d**.

purified on silica gel (SiO_2) column with chloroform-methanol solvent system as eluent. The reaction pathway of the phthalocyanine derivatives is shown in Scheme 2.

Metal-free phthalocyanine (**1a**) **(H_2Pc):** Green solid. Yield: 32 mg (16%). m.p.: >300 °C. IR (ATR) ν_{max}/cm^{-1}: 3292 (N − H), 3072 $(C\text{-}H)_{Ar}$, 3000–2838 $(C-H)_{Aliph}$, 1611–1585 (C=C), 1499, 1458, 1416, 1337, 1321, 1218, 1123, 1090, 1006, 924, 822, 744, 699. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.22 (s, 20H, Ar–H), 6.83–6.70 (m, 12H, =C-H and $=CH_2$), 3.91–3.56 (m, 24H, $O–CH_3$), 2.10–1.98 (m, 8H, $–CH_2$). UV-Vis ($CHCl_3$): λ_{max}, nm (log ε): 706 (5.05), 670 (4.98), 641 (4.16), 609 (4.30), 344 (4.44). MALDI-TOF-MS, (m/z): Calculated: 1283.40; Found: 1283.12 $[M]^+$.

Zinc(II) phthalocyanine (**1b**) **(ZnPc):** Turquoise solid. Yield: 33 mg (15.7%). m.p.: >300 °C. IR (ATR) ν_{max}/cm^{-1}: 3071 $(C\text{-}H)_{Ar}$, 2999–2837 $(C-H)_{Aliph}$, 1608–1588 (C=C), 1456, 1393, 1334, 1265, 1220, 1122, 1088, 1039, 942, 819, 744. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.29 (s, 12H, Ar–H), 6.68 (s, 8H, Ar-H), 5.29 (s, 12H, =C-H and $=CH_2$), 3.78–3.56 (m, 24H, $O–CH_3$), 1.58 (m, 8H, $–CH_2$). UV-Vis ($CHCl_3$: λ_{max}, nm (log ε): 685 (5.05), 617 (4.38), 351 (4.72). MALDI-TOF-MS, (m/z): Calculated: 1346.78; Found: 1346.22 $[M]^+$.

Cobalt(II) phthalocyanine (**1c**) **(CoPc):** Blue solid. Yield: 15 mg (7%). m.p.: >300 °C. IR (ATR) ν_{max}/cm^{-1}: 3071 $(C\text{-}H)_{Ar}$, 2932–2838 $(C-H)_{Aliph}$, 16,010–1591 (C=C), 1457, 1408, 1331, 1267, 1224, 1123, 1092, 1054, 956, 818, 750. UV-Vis ($CHCl_3$): λ_{max}, nm (log ε): 678 (5.04), 611 (4.47), 329 (4.78), 289 (4.85). MALDI-TOF-MS, (m/z): Calculated: 1340.3; Found: 1340.10 $[M]^+$.

Copper(II) phthalocyanine (**1d**) **(CuPc):** Blue solid. Yield: 43 mg (21.5%). m.p.: >300 °C. IR (ATR) ν_{max}/cm^{-1}: 3071 $(C\text{-}H)_{Ar}$, 2999–2837 $(C-H)_{Aliph}$, 1610–1590 (C=C), 1500, 1457, 1400, 1333, 1266, 1222, 1123, 1090, 1046, 947, 817, 745. UV-Vis ($CHCl_3$): λ_{max}, nm (log ε): 685 (5.02), 616 (4.39), 340 (4.66). MALDI-TOF-MS, (m/z): Calculated: 1344.95; Found: 1344.15 $[M]^+$.

2.2.4. Octa-peripheral eugenol-substituted phthalocyanines (2a–2d)

Synthesis and purification of octa-peripheral substituted phthalocyanine compounds (**2a**–**2d**) were similar to tetra-substituted phthalocyanine compounds (**1a**–**1d**). The mixture of phthalonitrile compound (**2**) (0.1 g, 0.19 mmol), 1,8-diazabicyclo[4.5.0]undec-7-ene (DBU) (10 drops), n-pentanol (3.0 mL), no metal salt for **2a** and 18 mg $Zn(CH_3COO)_2$ (for **2b**), 13 mg $CoCl_2$ (for **2c**) and 13 mg $CuCl_2$ (for **2d**) were heated to 160 °C and stirred for 24 h at this temperature. After cooling at room temperature, the reaction mixture was precipitated by the addition of ethanol and filtered off. All of the phthalocyanine compounds were purified with column chromatography by using silica gel and chloroform-methanol solvent system. The reaction pathway of the phthalocyanine derivatives is shown in Scheme 2.

Metal-free phthalocyanine (**2a**) **(H_2Pc):** Green solid. Yield: 10 mg (5%). m.p.: >300 °C. IR (ATR) ν_{max}/cm^{-1}: 3294 (N − H), 3070 $(C\text{-}H)_{Ar}$, 3002–2850 $(C-H)_{Aliph}$, 1588–1502 (C=C), 1451, 1416, 1341, 1239, 1207, 1125, 1016, 961, 839, 751. UV-Vis ($CHCl_3$): λ_{max}, nm (log ε): 704 (5.07), 671 (4.98), 640 (4.56), 610 (4.42), 349 (4.87). MALDI-TOF-MS, (m/z): Calculated: 2052.26; Found: 2052.79 $[M]^+$.

Zinc(II) phthalocyanine (**2b**) **(ZnPc):** Green solid. Yield: 37 mg (18.5%). m.p.: >300 °C. IR (ATR) ν_{max}/cm^{-1}: 3071 $(C\text{-}H)_{Ar}$, 2999–2840 $(C-H)_{Aliph}$, 1591–1502 (C=C), 1489, 1417, 1455, 1390, 1336, 1240, 1205, 1124, 1093, 990, 887, 869, 746. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.32 (s, 24H, Ar–H), 6.65 (m, 8H, =C-H), 5.29 (s, 16H, $=CH_2$), 3.59 (s, 48H, $O\text{–}CH_3$), 2.05 (s, 16H, $-CH_2$). UV-Vis ($CHCl_3$): λ_{max}, nm (log ε): 684 (5.00), 618 (4.30), 356 (4.65). MALDI-TOF-MS, (m/z): Calculated: 2115.64; Found: 2115.92 $[M]^+$.

Cobalt(II) phthalocyanine (**2c**) **(CoPc):** Blue solid. Yield: 8 mg (4%). m.p.: >300 °C. IR (ATR) ν_{max}/cm^{-1}: 3075 $(C\text{-}H)_{Ar}$, 2933–2839 $(C-H)_{Aliph}$, 1589–1502 (C=C), 1452, 1403, 1342, 1241, 1205, 1126, 1099, 1042, 1004, 961, 897, 753. UV-Vis ($CHCl_3$): λ_{max}, nm (log ε): 678 (5.00), 611 (4.39), 328 (4.75), 300 (4.82). MALDI-TOF-MS, (m/z): Calculated: 2109.19; Found: 2109.73 $[M]^+$.

Copper(II) phthalocyanine (**2d**) **(CuPc):** Green solid. Yield: 45 mg (22.5%). m.p.: >300 °C. IR (ATR) ν_{max}/cm^{-1}: 3075 $(C\text{-}H)_{Ar}$, 2999–2839 $(C-H)_{Aliph}$, 1589–1501 (C=C), 1449, 1389, 1341, 1240, 1205, 1126, 1096, 997, 891, 747. UV-Vis ($CHCl_3$): λ_{max}, nm (log ε): 686 (5.03), 617 (4.39), 341 (4.71). MALDI-TOF-MS, (m/z): Calculated: 2113.81; Found: 2113.33 $[M]^+$.

2.3. Determination of in vitro antibacterial and antifungal activities

2.3.1. Antibacterial activity

In this study, microdilution broth method with alamar blue was used to determine the MIC values of the compounds *in vitro* against nine selected standard bacteria and one yeast isolate.

2.3.2. Preparation of standard bacterial isolates

The antimicrobial activities of the compounds against the standard bacterial and yeast isolates were evaluated using in the microplates using the microdilution broth method with the addition of alamar blue (Performance Standards for Antimicrobial Susceptibility Testing). The standard obtained from the American Type Culture Collection (ATCC); *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 700603, *Shigella flexneri* ATCC 12022, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* (MRSA) ATCC 43300, *Acinetobacter baumannii* ATCC 19606, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644 and *Candida albicans* ATCC 14053 strains were used. Standard bacterial strains were produced by incubating overnight at 37 °C using Blood agar (Merck) and Eosin Methylene Blue Agar (EMB, Merck) media and purity checks were performed. Sabouraud Dextrose Agar medium was used for yeast isolate. Standard bacterial isolates were prepared by diluting them in sterile saline to approximately 1.5 × 108 cfu/mL according to McFarland 0.5 turbidity chart.

2.3.3. In vitro antimicrobial activity test

Antimicrobial activity testing of the compounds was performed using 96-well sterile microplates. The stock solutions of the compounds were first dissolved with DMSO, then distilled water was added to a concentration of 20 mg/mL and sterilized by filtration through a 0.45-μm pore diameter sterile membrane filter. One hundred microlitres of Mueller Hinton Broth medium was added to all wells for antibacterial activity test; Sabouraud Dextrose Broth medium was used for antifungal activity test. The dilutions of the compounds as previously described [26, 27] were performed between 5000 μg/mL and 156 μg/mL. One hundred microlitres of standard bacterial or yeast suspensions were added to the wells. Negative and positive control wells were added to the microplate and the microplates were incubated in an incubator set at 37 °C. After 20 h of incubation, 20 μL of alamar blue (resazurin) was added to the wells and incubated for another 4 h. Microplates were evaluated visually after 24 and 48 h. Indicator alamar blue color in the wells turned to pink, bacteria or yeast growth continued, the absence of color change was interpreted as stopping of bacterial growth. Samples taken from each well with the loop as a reproduction control were inoculated on the Blood agar medium and the reproduction was checked. While the antimicrobial activity test for each compound was repeated twice, Amikacin and Amphotericin B were used as control drugs.

3. Results and discussion

3.1. Synthesis and characterization

Phthalonitrile compounds **1** and **2** were obtained by nucleophilic substitution of 4-allyl-2,6-dimethoxyphenol with 4-nitrophthalonitrile for **1** and 4,5-dichloro-phthalonitrile for **2**

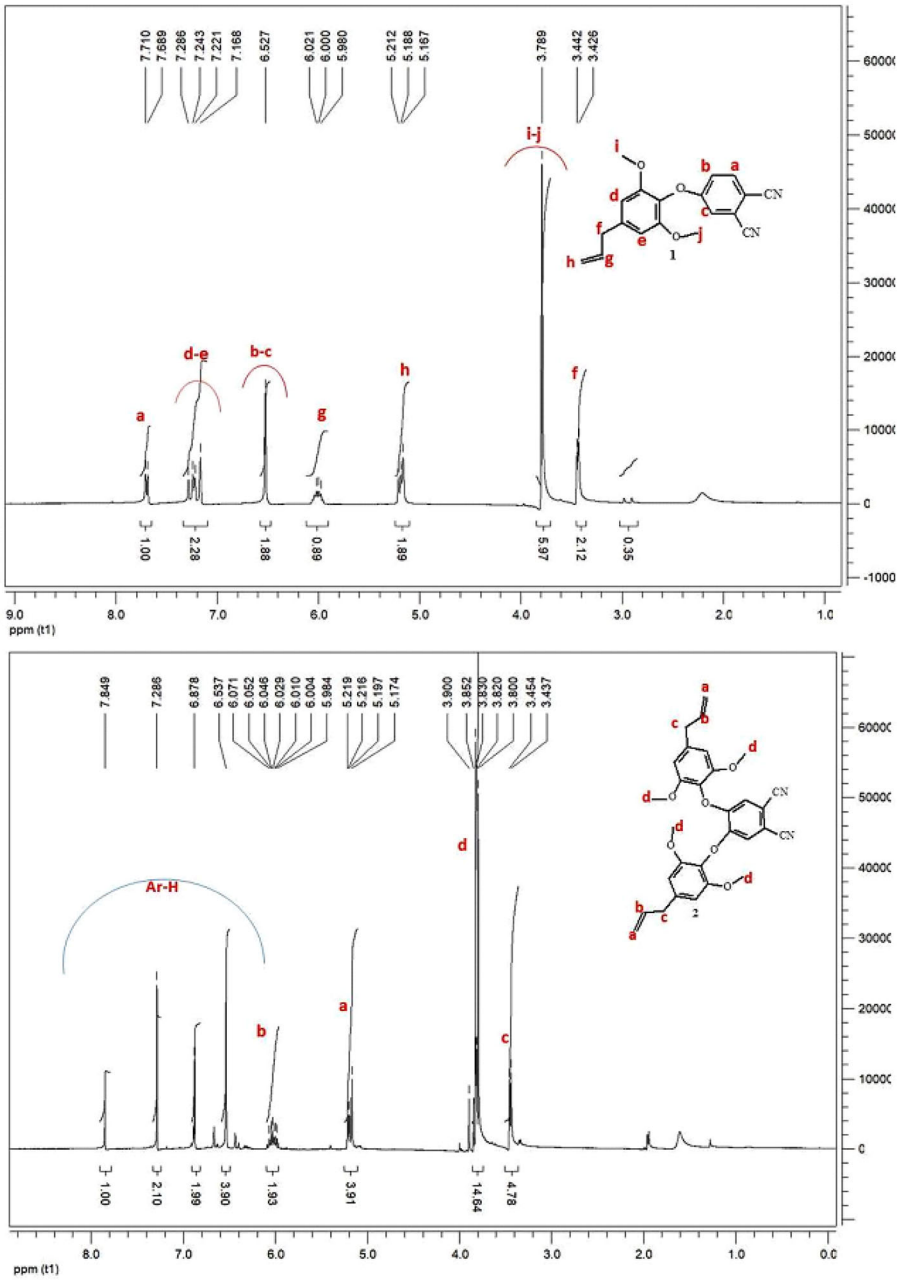


Figure 1. ^{1}H NMR spectrum of phthalonitrile compounds **1** and **2**.

in the presence of K_2CO_3 as catalyst at 60 °C and 90 °C, in DMF and acetonitrile, respectively (Scheme 1). The synthesis of peripherally tetra- and octa-H_2Pc, zinc(II), cobalt(II) and copper(II) phthalocyanines with eugenol group have been performed by the procedure described in Scheme 2. The reaction yields for **1** and **2** were 85% and 36% after column chromatography. The structure of **1** and **2** were determined by ^{1}H and ^{13}C NMR spectroscopy and further confirmed by FT-IR and mass spectroscopy.

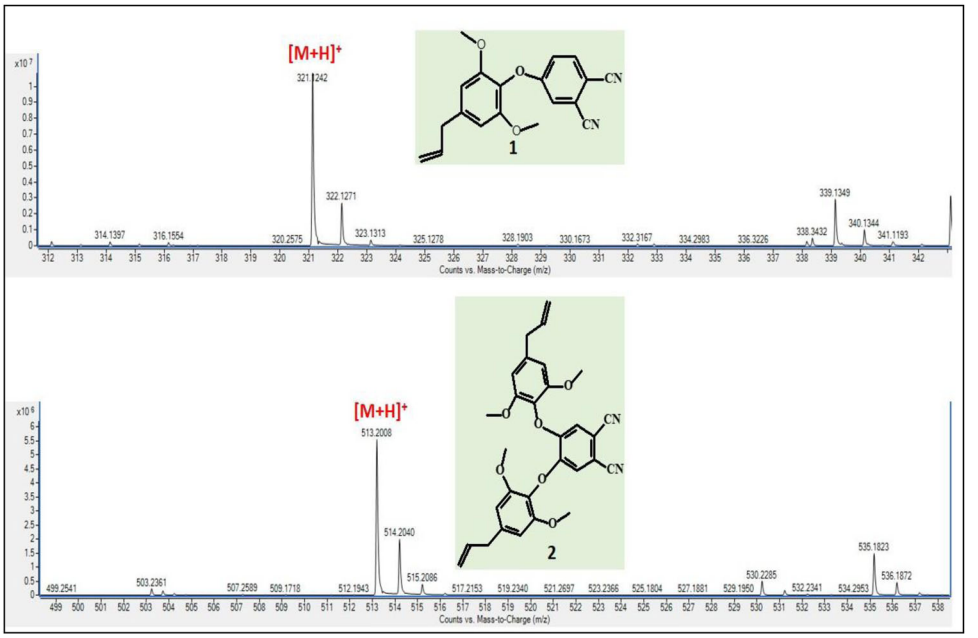


Figure 2. MS spectra of phthalonitrile compounds **1** and **2**.

In the FT-IR spectra of phthalonitrile compounds **1** and **2**, the disappearance of the OH groups (3500 cm^{-1}) of methoxy-eugenol (4-allyl-2,6-dimethoxyphenol) compound and the presence of characteristic stretching bands for C≡N groups at 2229, 2233 cm^{-1} were indicative of successful coupling. Also, formation of **2** was confirmed by the disappearance of the C–Cl band of 1,2-dichloro-4,5-dicyanobenzene at 640 cm^{-1} in the IR spectrum. Other IR stretching vibrations of **1** and **2** were similar to the compound of 4-allyl-2,6-dimethoxyphenol **1**. Additionally characteristic vibration peaks were observed for **1** and **2**: aromatic C–H stretches at 3083 cm^{-1} (for **1**) and 3079 cm^{-1} (for **2**), aliphatic C–H stretches at 3011–2840 cm^{-1} (for **1**) and 3032–2842 cm^{-1} (for **2**) and allyl (C=C) group stretches of at 1601–1588 cm^{-1} (for **1**) and 1599–1502 cm^{-1} (for **2**).

In the ^{1}H NMR spectra of **1** and **2**, the OH group at 10.8 ppm disappeared, aromatic and aliphatic protons appeared at around 7.84–5.16 ppm and 3.90–3.42, respectively (Figure 1). The ^{13}C NMR spectra of **1** and **2** indicated the presence of nitrile carbon atoms (C≡N) at 115.70 and 115.30 ppm (for **1**) and 116.63 ppm (for **2**), methoxy carbon atoms (O–CH_3) at 56.08 pm (for **1**) and 56.23 ppm (for **2**) and methyl carbon atoms (–CH_2) at 40.68 ppm (for **1**) and 40.67 ppm (for **2**), respectively (supplementary information Figures S1 and S2). The mass spectra of **1** and **2** confirmed the structures proposed, with the molecular ions at 321.24 $[M+H]^+$ for **1** and 513.20 $[M+H]^+$ for **2** (Figure 2).

4-Substituted phthalonitrile derivative **1** was used to synthesize peripheral tetra-substituted metal-free (H_2Pc) **1a** and metallophthalocyanines **1b**–**1d**. The peripherally octa-substituted H_2Pc **2a** and metallophthalocyanines **2b**–**2d** were accomplished by cyclotetramerization of 4,5-disubstituted phthalonitrile derivative **2**. While, the metal-

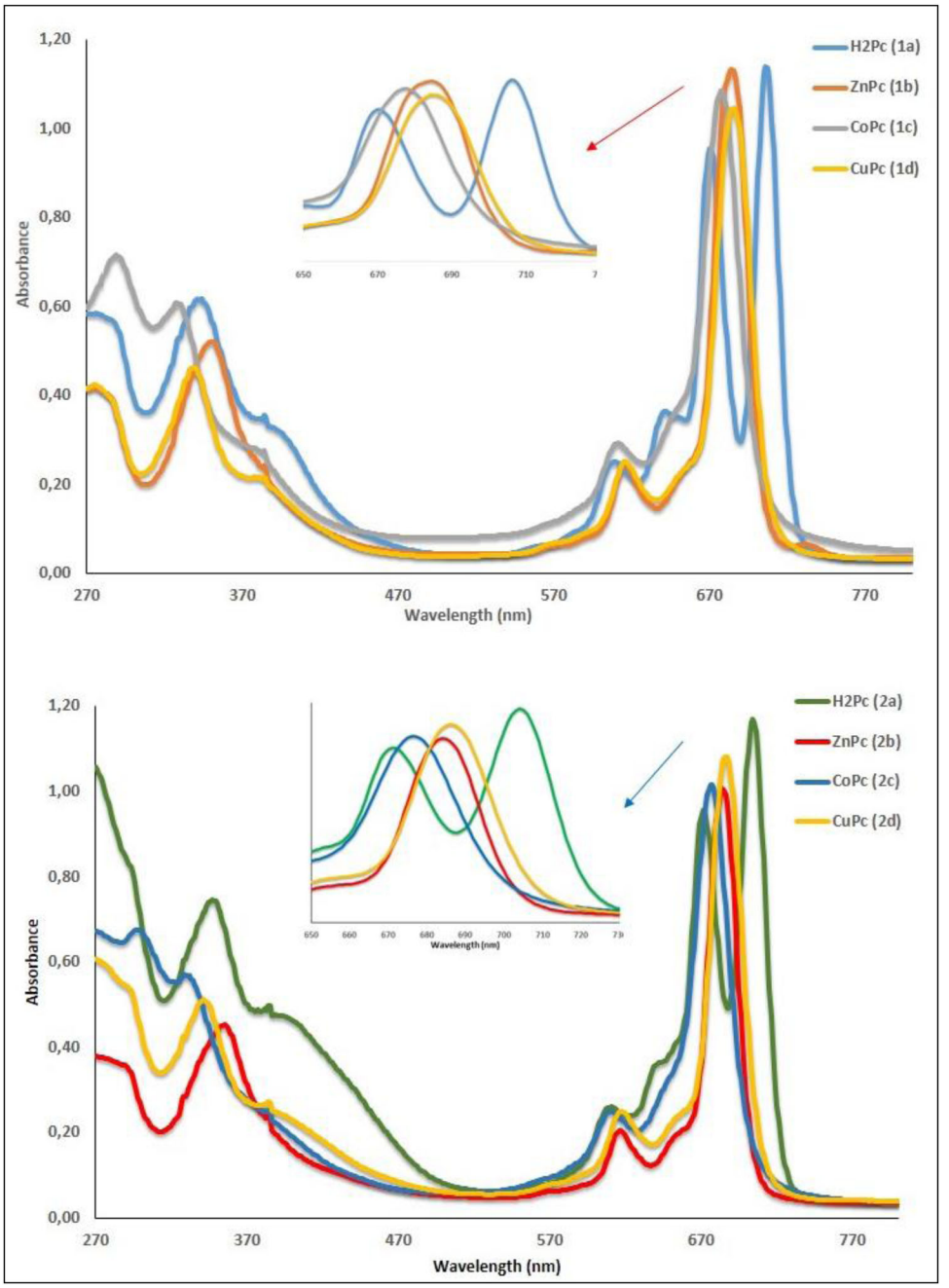


Figure 3. UV-Vis absorption spectra of peripheral tetra-substituted phthalocyanines **1a**–**1d** and octa-substituted phthalocyanines **2a**–**2d** in $CHCl_3$ at 1×10^{-5} M.

free phthalocyanines (H_2Pcs) **1a** and **2a** were obtained in the presence of DBU in *n*-pentanol, the Zn, Co and Cu Pcs **1b**–**d** and **2b**–**d** were obtained from corresponding metal salts, $Zn(CH_3COO)_2$, $CoCl_2$ or $CuCl_2$, in *n*-pentanol and using DBU as the catalyst. The synthesized Pcs were purified by column chromatography using a silica gel (SiO_2)

column with chloroform-methanol as the eluent. Solubility is an important factor for many application areas. Novel synthesized MPcs (**1a**–**2d**) are highly soluble in common organic solvents such as chloroform, DMF, DMSO and THF.

In the IR spectra of metal-free phthalocyanines **1a** and **2a** showed presence of the –NH vibration bands at 3292 cm^{-1} for **1a** and 3294 cm^{-1} for **2a** and the disappearance of the C≡N vibration bands at 2233 cm^{-1} are examined as evidence of the formation of H_2Pc **1a** and H_2Pc **2a**. Disappearance of the C≡N vibrations bands at 2233 cm^{-1} are considered as evidence of the formation of zinc(II) Pcs **1b** and **2b**, cobalt(II) Pcs **1c** and **2c** and copper(II) Pcs **1d** and **2d**.

In the mass spectra of the metal-free, zinc, cobalt and copper Pcs, the presence of molecular ion peaks at m/z = 1283.12 $[M]^+$ for **1a**; 1346.22 $[M]^+$ for **1b**; 1340.10 $[M]^+$ for **1c**; 1344.15 $[M]^+$ for **1d**; 2052.79 $[M]^+$ for **2a**; 2115.92 $[M]^+$ for **2b**; 2109.73 $[M]^+$ for **2c** and 2113.33 $[M]^+$ for **2d** confirmed the proposed structures (see supplementary information).

3.2. Absorption properties of phthalocyanine compounds (1a–2d)

Phthalocyanine compounds **1a**–**2d** showed two strong absorption bands in electronic spectra. One is in the visible region at 706 nm (Q-band) and the other is in the UV region at 356 nm (B-band). UV-vis spectra of the peripheral tetra- metal-free phthalocyanine **1a** and its octa- derivative **2a** were measured at chloroform (Figure 3). According to these spectra, a siplet Q-band along with two shoulders were observed. The split Q-band is characteristic of metal-free phthalocyanines and these bands were observed at 706 nm and 670 nm with two shoulders at 641 nm and 609 nm for **1a**; 704 nm and 671 nm and shoulders at 640 nm and 610 nm for **2a**. These results present that metal-free Pcs with D_{2h} symmetry show two intense absorption bands around 700 nm because of the conjugated 18-π electron system (π-π* transition), as expected [28]. Peripheral octa-substituted metal-free Pc **2a** showed a blue-shift (*ca.* 2 nm) Q-band when compared to the peripheral tetra-substituted metal-free Pc **1a**. The intensity of the Q-band of metal-free phthalocyanines **1a** and **2a** followed the order Octa-H_2Pc (**2a**) > Tetra-H_2Pc (**1a**) (Figure 3).

The UV-Vis absorption spectra of the synthesized ZnPcs (**1b**–**2b**), CoPcs (**1c**–**2c**) and CuPcs (**1d**–**2d**) in $CHCl_3$ at 1×10^{-5} M concentration are shown in Figure 3. The UV-Vis absorption spectra showed Q-band absorptions at 685/617 nm for **1b** (corresponding to degenerate D_{4h} symmetry), 684/618 nm for **2b**, 678/611 nm for **1c**, 678/611 nm for **2c**, 685/616 nm for **1d** and 686/617 nm for **2d**, while the B-band absorptions were observed at 351, 356, 329/289, 328/300, 340 and 341 nm, respectively.

Metallophthalocyanines **1b**, **1c** and **1d** have the same peripheral but have different metal ions in the core. Their Q-band positions were similar due to the same substituent on their peripheries. However, CoPc **1c** showed a blue-shift (*ca.* 7 nm) Q-band when compared to the other phthalocyanine complexes **1b** and **1d**. Also, as shown in Figure 3, octa-substituted metallophthalocyanines **2b**, **2c** and **2d**, having the same substituted group but containing different metal atoms in the core, showed similar Q-band positions in the UV-Vis spectra. CoPc **2c** showed a blue-shift (*ca.* 7 nm) Q-band

Table 1. Minimum inhibitory concentration (MIC) values of all compounds against standard bacteria and fungi.

	MIC values (μg/mL)								
Bacteria and fungi	**1**	**1a**	**1b**	**1c**	**1d**	**2**	**2b**	**2c**	**2d**
E. coli	>5000	>5000	>5000	>5000	>5000	>5000	>5000	5000	>5000
S. typhimurium	>5000	>5000	>5000	>5000	>5000	>5000	5000	2500	5000
S. flexneri	5000	5000	5000	1250	5000	>5000	5000	312	1250
A. baumannii	>5000	>5000	>5000	>5000	>5000	5000	>5000	>5000	>5000
MRSA	>5000	>5000	>5000	>5000	>5000	1250	>5000	1250	>5000
P. aeruginosa	>5000	>5000	5000	5000	5000	>5000	5000	1250	5000
K. pneumoniae	>5000	>5000	>5000	>5000	>5000	>5000	>5000	1250	>5000
E. faecalis	>5000	>5000	2500	>5000	5000	>5000	>5000	5000	>5000
L. monocytogenes	>5000	>5000	2500	>5000	5000	>5000	5000	>5000	>5000
C. albicans	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000	>5000

MRSA, methicillin-resistant *S. aureus*.

when compared to **2b** and **2d**. Compound **2d** showed a slight shift (*ca.* 2 nm) to the lower energy side in comparison of **2b** in the UV-Vis absorption spectrum.

Phthalocyanine compounds **2a**–**d** became highly soluble in various organic solvents, including $CHCl_3$, THF and DMF, due to the incorporation of eight peripheral 4-allyl-2,6-dimethoxyphenol groups into the phthalocyanine rings. The Q-band positions of phthalocyanines bearing four peripheral 4-allyl-2,6-dimethoxyphenol groups (**1a**–**1d**) and bearing eight peripheral 4-allyl-2,6-dimethoxyphenol groups (**2a**–**2d**) into the phthalocyanine rings were very similar. However, CuPc **2d** showed a red-shift (686 nm) Q-band when compared to all of the metallophthalocyanine complexes **1b**, **2b**, **1c**, **2c** and **1d**.

The intensity of the Q-band of metallophthalocyanines **1b**–**2d** followed the order T-ZnPc (**1b**) > T-CoPc (**1c**) > O-CuPc (**2d**) > T-CuPc (**1d**) > O-CoPc (**2c**) > O-ZnPc (**2b**).

When comparing the absorption properties of new eugenol-substituted phthalocyanine compounds in literature and our works, it is inferred that the methoxy-eugenol substituted Pcs showed absorption at higher wavelength in the UV-Vis spectroscopy [16, 29].

3.3. In vitro antibacterial activity studies

While some of the nine compounds synthesized were found to have highest concentrations of antibacterial activity against standard bacterial isolates, some compounds were found to be effective at the low concentrations studied. However, no compound has antifungal activity against yeast isolate. The antibacterial activity images of the most effective compound (**2c**) against bacterial isolates are shown in supplementary information Figure S6. The MIC values of the compounds are given in Table 1. Compound **2c** was found to be effective against all bacterial strains except for *L. monocytogenes* (supplementary information Figure S6).

All compounds (**1**–**2d**) were found to have antibacterial activity against at least one bacterium, albeit at high concentrations. Compound **2c**, which affects the most bacteria, showed different levels of antibacterial activity against *S. flexneri*, *S. aureus* (MRSA), *P. aeruginosa*, *K. pneumoniae*, *S. typhimurium*, *E. coli*, *A. baumannii* and *E. faecalis* and MIC values were determined as 312, 1250, 1250, 1250, 2500, 5000, 5000 and 5000 μg/mL, respectively. The least affected bacteria were *E. coli*. Compounds **1** and

1a were found to be effective only on one bacterium (*S. flexneri* MIC: 5000 μg/mL). Lastly, the connected substituent on Pc centrally located metal atom and geometry of the Pc may affect antimicrobial activity.

Antimicrobial activity studies of phthalocyanine complexes are rare in the literature. In the previous study in 2019, antimicrobial activities of phthalocyanine compounds containing morpholine group were studied and peripheral tetra- CoPc (MIC=around 625–10,000 μg/mL) was found to have higher activities than other Pcs [9]. When compared with this study, it was seen that the synthesized octa-CoPc (**2c**) complex was more active. In the literature, there are quite a few studies about eugenol-substituted phthalocyanine. Some of them are on DNA-interaction, catalytic activity, enzyme inhibition, electrochemical and spectroelectrochemical. Antimicrobial activity studies of eugenol-substituted phthalocyanine complexes are not in the literature. There are a few studies in which antibacterial effect of metallophthalocyanine containing eugenol on cotton fabric has been studied. Ozguney and co-workers investigated antibacterial effect of metallophthalocyanine (ZnPc) containing eugenol printed on cotton fabric. The samples were tested against two types of bacteria, *S. aureus* (Gram-positive) and *K. pneumoniae* (Gram-negative). It was observed that it had an effective antibacterial action to *S. aureus* but not effective on *K. pneumoniae* [30]. Ozdemir and co-workers investigated the effect of eugenol group substitutions (tetra and octa) on antibacterial properties of metallophthalocyanines (M: Zn, Cu, Co, Ni) printed on cotton fabric. All synthesized phthalocyanine pigments exhibited various levels of antibacterial activity against *S. aureus* and *K. pneumoniae* bacteria on cotton fabric [25]. There is no other study in the literature where we can compare antimicrobial activity of new compounds. In this study microdilution broth method with alamar blue was used to determine the MIC values of the compounds *in vitro* for the antimicrobial effects of synthesized new compounds. In this sense, this study reached its purpose and contributed to the literature.

4. Conclusion

We synthesized and characterized novel phthalonitrile derivatives **1** and **2**. Afterward, we synthesized peripheral tetra- and octa-substituted H_2- **1a** and **2a**, Zn(II) **1b** and **2b**, Co(II) **1c** and **2c** and Cu(II) **1d** and **2d** phthalocyanines which are substituted with 4-allyl-2,6-dimethoxyphenol (methoxyeugenol). The structures of the synthesized phthalocyanines **1a**–**2d** have been fully characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, MALDI-TOF-MS and UV-Vis spectroscopy. Finally, all synthesized compounds (**1**–**2d**) were subjected to *in vitro* antibacterial and antifungal activity studies. According to the test results, octa- cobalt phthalocyanine complex **2c** was found to be highly effective in terms of antibacterial activity (MIC = 312 μg/mL) and it was concluded that further studies could be continued as a drug candidate for this compound. Compound **2c** showed antibacterial activity against all bacteria studied except *L. monocytogenes*. In light of these results, it was concluded that **2c** may be drug precursors. In order to use the synthesized compounds as a drug candidate, control studies should be performed in experimental animal models *in vivo*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] N.B. McKeown. *Phthalocyanine Materials: Synthesis Structure and Function*, Cambridge University Press, Cambridge (1998).
[2] C.C. Leznoff, A.B.P. Lever (Eds.). *Phthalocyanines: Properties and Applications*, Vols. **1–4**, VCH, New York (1996).
[3] A. Suzuki, H. Okumura, Y. Yamasaki, T. Oku. *Appl. Surf. Sci.*, **488**, 586 (2019).
[4] A. Koca, A. Kalkan, Z.A. Bayır. *Electrochim. Acta*, **56**, 5513 (2011).
[5] J. Riquelme, K. Neira, J.F. Marco, P. Hermosilla-Ibáñez, W. Orellana, J.H. Zagal, F. Tasca. *Electrochim. Acta*, **265**, 547 (2018).
[6] P. Sen, M. Managa, T. Nyokong. *Inorg. Chim. Acta*, **491**, 1 (2019).
[7] A. Aktas Kamiloglu, E.T. Saka, I. Acar, K. Tekintas. *J. Coord. Chem.*, **72**, 2778 (2019).
[8] D.D. Erbahar, M. Harbeck, G. Gümüş, I. Gürol, V. Ahsen. *Sens. Actuat. B: Chem.*, **190**, 651 (2014).
[9] D. Unluer, A. Aktas Kamiloglu, S. Direkel, E. Bektas, H. Kantekin, K. Sancak. *J. Organomet. Chem.*, **900**, 120936 (2019).
[10] H.R. Pekbelgin Karaoğlu, A. Gül, M.B. Koçak. *Dyes Pigm.*, **76**, 231 (2008).
[11] B. Ertem, H. Yalazan, Ö. Güngör, G. Sarkı, M. Durmuş, E.T. Saka, H. Kantekin. *J. Lumin.*, **204**, 464 (2018).
[12] Z. Biyiklioglu, H. Alp. *Dyes Pigm.*, **132**, 213 (2016).
[13] I. Gürol, M. Durmuş, V. Ahsen, T. Nyokong. *Dalton Trans.*, **34**, 3782 (2007).
[14] C. Kantar, Z.S. Şahin, O. Büyükgüngör, S. Şaşmaz. *J. Mol. Struct.*, **1089**, 48 (2015).
[15] C. Kantar, F. Mert, S. Şaşmaz. *J. Organomet. Chem.*, **696**, 3006 (2011).
[16] S. Şaşmaz, E. Ağar, A. Ağar. *Dyes Pigm.*, **42**, 117 (1999).
[17] A. Marchese, R. Barbieri, E. Coppo, I.E. Orhan, M. Daglia, S.F. Nabavi, M. Izadi, M. Abdollahi, S.M. Nabavi, M. Ajami. *Crit. Rev. Microbiol.*, **43**, 668 (2017).
[18] K. Pramod, M.R. Aji Alex, M. Singh, S. Dang, S.H. Ansari, J. Ali. *J. Drug Target.*, **24**, 24 (2016).
[19] A. Martínez-Herrera, A. Pozos-Guillén, S. Ruiz-Rodríguez, A. Garrocho-Rangel, A. Vértiz-Hernández, D.M. Escobar-García. *Mediat. Inflamm.*, **2016**, 1 (2016).
[20] Q. Jiang, J. Chen, C. Yang, Y. Yin, K. Yao. *Biomed. Res. Int.*, **2019**, 1 (2019).
[21] P.S. Yadav, D. Prakash, G.P. Senthilkumar. *Int. J. Pharm. Sci. Drug Res.*, **3**, 1 (2011).
[22] N. Sehlotho, T. Nyokong. *J. Mol. Catal. A: Chem.*, **209**, 51 (2004).
[23] E.T. Saka, R.Z. Uslu Kobak, H. Alp, G. Sarkı, A. Koca, H. Kantekin. *Synth. Met.*, **217**, 295 (2016).
[24] E.T. Saka, G. Sarkı, H. Kantekin, A. Koca. *Synth. Met.*, **214**, 82 (2016).
[25] P. Saral Ozdemir, A.T. Özgüney, G. Kaya Kantar, S. Şaşmaz. *AATCC J. Res.*, **4**, 34 (2017).
[26] N.O. İskeleli, Y.B. Alpaslan, Ş. Direkel, A.G. Ertürk, N. Süleymanoğlu, R. Ustabaş. *Spectrochim. Acta, Part A.*, **139**, 356 (2015).
[27] M. Er, A. Ozer, Ş. Direkel, T. Karakurt, H. Tahtaci. *J. Mol. Struct.*, **1194**, 284 (2019).
[28] A. Aktaş Kamiloğlu, D. Akyüz, A. Koca, İ. Acar. *J. Incl. Phenom. Macrocycl. Chem.*, **92**, 223 (2018).
[29] M.S. Agırtas, C. Karatas, S. Gumuş. *J. Inorg. Gen. Chem.*, **641**, 1334 (2015).
[30] A.T. Ozguney, C. Kantar, P. Saral, N. Seventekin, S. Sasmaz. *Tekstil ve Konfeksiyon*, **23**, 261(2013).