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RESEARCH ARTICLE

Synthesis, Characterization and Antioxidant and Antimicrobial Properties of New Ester and Amide Derivatives of Indole-2-Carboxylic Acid

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Synthesis, Characterization and Antioxidant and Antimicrobial Properties of New Ester And Amide Derivatives of Indole-2-Carboxylic Acid

Summary

New ester and amide derivatives of indole-2-carboxylic acid (1-6) were synthesized and evaluated for their in vitro antioxidant and antimicrobial properties. The compounds 5 and 6 demonstrated scavenging effect against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. These two compounds showed excellent reducing power at 200, 150, 100 and 50 µg/ml concentrations as compared to butylated hydroxytoluene (BHT). All tested compounds also exhibited more powerful Fe^{2+} chelating activity than Ethylenediaminetetraacetic acid (EDTA). With regard to antimicrobial properties, compound 2 was most active derivative against Enterococcus faecalis and also demonstrated significant activity against Candida albicans with a MIC value of 8 µg/mL. Compounds 1, 3, 5 and 6 had noticeable antifungal activities against C. albicans.

Key Words: *Indole, DPPH, reducing power, chelating activity, antimicrobial activity*

İndol-2-karboksilik Asitin Yeni Ester ve Amit Türevlerinin Sentezi, Yapı Aydınlatması ve Antioksidan and Antimikrobiyal Özellikleri

Özet

İndol-2-karboksilik asitin yeni ester ve amit türevleri (1-6) sentezlendi ve in vitro antioksidan ve antimikrobiyal özellikleri incelendi. Bileşik 5 ve 6 2,2-diphenyl-1-pikrilhidrazil (DPPH) radikallerine karşı süpürücü etki göstermiştir. Bu iki bileşik 200, 150, 100 and 50 µg/ml konsantrasyonlarda butil hidroksitoluen (BHT) ile karşılaştırıldığında mükemmel indirgeyici güç göstermiştir. Test edilen tüm bileşikler etilendiamintetraasetik asit (EDTA)'ten daha güçlü Fe^{2+} ile şelat yapıcı etki göstermiştir. Antimikrobiyal özellikleri ile ilgili olarak, bileşik 2 E. faecalis'e karşı en aktif türevdir ve 8 µg/ml MIK değeri ile C. albicans'a karşı da önemli bir aktivite göstermiştir. Bileşik 1, 3, 5 ve 6 C. albicans'a karşı belirgin antifungal aktiviteye sahip bulunmuştur.

Anahtar Kelimeler: *indol, DPPH, redükleyici güç, kelat yapıcı etki, antimikrobiyal aktivite*

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INTRODUCTION

Free radicals are continuously produced as side products through enzymatic reactions in normal metabolic pathways. Sometimes these free radical side products penetrate through active sides of enzymes and reacting with molecular oxygen, form free oxygen radicals. Reactive oxygen species (ROS) formed in the cells can be removed through "antioxidant defense systems" or "antioxidants" in short. When there are more ROS produced then the ones removed by the cellular defense system, it is defined as oxidative stress. It is thought that oxidative stress, cell injuries caused by free oxygen radicals; contribute in complications for several chronic diseases. In cases such as atherogenesis, emphysema/bronchitis, Parkinson disease, Duchenne type muscular dystrophy, preeclampsia in pregnancy, cervix cancer, liver disease (hepatopathy) due to alcolism, hemodialysis, diabetes mellitus, acute renal failure, Down's syndrome, aging, retrolental fibroplasia, cerebrovascular disorders, ischemia/reperfusion injuries, oxidative stress is considered to play a role in their pathogenesis (1, 2).

As the role of oxidative stress in the pathogenesis of the diseases is understood, studies were focused on this area. In oxidative stress works, increases in free radicals or inadequacy of antioxidant defense systems are investigated. For this reason, methods suitable for analysis of several materials such as serums, erythrocytes and cell samples have been developed (3, 4).

Indole derivatives are very common in the body and the diet, and synthetic and natural products containing indole moieties are an important class of therapeutic agents (5, 6). Tryptamin, serotonin, methoxytryriptamin, melatonin, indoleacetic acid, indolepropionic acid, L-tryptophan and the tetrahydro-beta-carboline indol alkaloids are draw attention as radical scavengers and anti-oxidant compounds. In the last decades, antioxidant activity of synthetic indole derivatives and their possible activity mechanisms have been studied. The antioxidant profiles of congeners at the 2nd or 3rd positions of the indole ring were found to be similar. Indole structure especially substitution of 2-position of indole influence the antioxidant efficacy in biological systems (7). Indole-2-carboxamide derivatives are stronger inhibitors for lipid peroxidation than indole-3-carboxamides. N-Substituted indole-2/3-carboxamide and also ester derivatives show potent antioxidant activity against superoxide radical (8-10). Inhibitor effect on lipid peroxidation of 2-phenyl indole derivatives was found as potent as BHT and compounds bearing electron-withdrawing groups showed the highest reduction in lipid peroxidation values (11).

Since the introduction of penicillin in the 1940s, antibiotics have had a history of success in controlling morbidity due to infectious diseases. But, as a consequence of frequent use, bacterial resistance to known classes of antibiotics has become a severe global problem in recent years and presents a continuous clinical challenge (12-14). Thus, an urgent need for new potent classes of antibiotics with novel modes of action persists.

Some indole-containing compounds were known to have antibacterial activities (15). Cumarin containing indole derivatives exhibited good to excellent in vitro activities against *Staphylacoccus aureus* and *E. faecium* including drug-resistant Gram-positive bacterial pathogens methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (16). Bis(indole) alkaloids were found as important key structures for the treatment of *S. aureus* infections via inhibition of sortase A (SrtA) activity (15). In addition, 1*H*-indole-4,7-dione derivatives showed potent antifungal activity against *Candida krusei*, *Candida neoformans*, and *Aspergillus niger* (17). Substituted pyrazino[1,2-a]indole was found to have antibacterial activity against pathogenic strains of *S. aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Streptomyces thermonitrificans* and *Escherichia coli* (18). Moreover, indole-substituted 2,5-dihydro-1H-2,5-pyrrolediones were found to have antibacterial activity against resistant strains of *S. aureus*, *Mycobacterium smegmatis* and some other Gram positive bacteria and it is found that the activity of compounds against *S. aureus* and *M. smegamtis*

improves by increasing hydrophobic properties as well as by hydrogen bond acceptors, depending on their distance from the indole-2-position (19). Ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylate derivatives display a variety of biological effects, such as antiviral effect, immunostimulative effect, and interferon-induced activity. In support of these findings, ethyl 1*H*-indole-3-carboxylate derivatives examined for their anti-HCV activities and found as promising leads (20).

In view of the above observations, in this study, some of ester and amide derivatives indole-2-carboxylic acid were prepared in order to evaluate their *in vitro* antioxidant properties by determining DPPH free radical scavenging activity and measuring their ability of chelating on metal ions and reducing power. Antimicrobial activities of title compounds were also tested using micro dilution method.

EXPERIMENTAL

Chemistry

The chemical reagents used in synthesis were purchased from Sigma (Germany) and Aldrich (US). Mueller Hinton Agar (MHA) (Merck), Mueller Hinton Broth (MHB) (Merck), Sabouraud Dextrose Agar (SDA) (Merck), RPMI-1640 medium with L-glutamine (Sigma), 3-[N-morpholino]-propansulfonic acid (MOPS) (Sigma), 96 well microplates (Falcon), transfer pipette (Biohit), ciprofloxacin (Sigma), fluconazole (Nobel), dimethylsulphoxide (DMSO) (Riedel de Haen) were used for antimicrobial study. The reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel plate (60 F254, 0.25 mm), and TLC plates were visualized by fluorescence quenching under UV light (254 nm). Melting points were determined by an SMP-II Digital Melting Point Apparatus and are uncorrected. ^{1}H-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 400 MHz FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of the Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm). IR spectra were obtained using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory. High resolution mass spectra data (HRMS) were collected using a Waters LCT Premier XE Mass Spectrometer operating in ESI (+) method, also coupled to an AQUITY Ultra Performance Liquid Chromatography system.

General procedure for the synthesis of 1*H*-indole-2-carboxylic acid ester and amide derivatives 1-6

To a solution of indole-2-carboxylic acid (1 mmol) and phenol or amine derivative (1 mmol) in dichloromethane (DCM) (10 mL), 4-Dimethylaminopyridine (DMAP) (0.2 mmol) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) (1.1 mmol) were added and the resulting solution was stirred overnight at room temperature. The reaction mixture was quenched with 0.5 N HCl and extracted with DCM. The organic phase was washed with a 1% $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under vacuum. The residue was purified by flash column chromatography (Combiflash®Rf) using DCM-MeOH as eluents.

4-Isopropylphenyl 1*H*-indole2-carboxylate 1

Elution with DCM-MeOH (0%-10%) yielded **1** as a white solid (yield 37%); mp 172-174 °C; IR (FTIR/FTNIR-ATR): 1705 cm^{-1} (C=O), 3351 cm^{-1} (N-H); ^{1}H-NMR ($CDCl_3$) δ: 9.06 (1H, s, indole H-1), 7.74 (1H, d, *J*=7.6 Hz, indole H-4), 7.45-7.34 (3H, m, indole H-6, phenyl H-2,6), 7.30-7.25 (4H, m, indole H-5,7, phenyl H-3,5), 7.18 (1H, m, indole H-9), 2.98-2.91 (1H, m, C<u>H</u>$(CH_3)_2$), 1.28 (3H, s, CH_3), 1.26 (3H, s, CH_3); HRMS $C_{18}H_{18}NO_2$ $[M+H]^+$ *Calc.*. 280.1338, Found m/z 280.1342.

4-Cyclopentylphenyl 1*H*-indole-2-carboxylate 2

Elution with DCM-MeOH (0%-10%) obt yielded ained **2** as a white solid (yield 45%); mp 210.2-212 °C; IR (FTIR/FTNIR-ATR): 1692 cm^{-1} (C=O), 3346 cm^{-1} (N-H); ^{1}H-NMR ($CDCl_3$) δ: 9.06 (1H, s, indole H-1), 7.74 (1H, d, *J*=8 Hz, indole H-4), 7.45-7.16 (4H, m, indole H-3,5,6,7,), 7.31 (2H, d, *J*=8.4 Hz, phenyl H-2,6), 7.16 (2H, d, *J*=8.4 Hz, phenyl H-3,5), 3.05-2.98 (1H, m, cyclopentyl H-1), 2.12-1.55 (8H, m, cyclopentyl H-2,3,4,5); HRMS $C_{20}H_{20}NO_2$ $[M+H]^+$ *Calc*. 306.1494, Found m/z 306.1494.

***N*-Cyclopropyl-1*H*-indole-2-carboxamide 3**
Elution with DCM-MeOH (0%-10%) yielded **3** as a white solid (yield 63%); mp 199-201 °C; IR (FTIR/FTNIR-ATR): 1614 cm^{-1} (C=O), 3279 cm^{-1} (N-H); ^{1}H-NMR ($CDCl_3$) δ: 9.45 (1H, s, indole H-1), 7.63 (1H, d, *J*=8.4 Hz, indole H-4), 7.45 (1H, m, indole H-7), 7.30-6.79 (3H, m, indole H-3,5,6,), 6.35 (1H, s, NH), 2.92 (1H, m, cyclopropyl H-1), 0.93-0.66 (4H, m, cyclopropyl H-2,3). HRMS $C_{12}H_{13}N_2O$ $[M+H]^+$ *Calc*. 201.1028, Found m/z 201.1027.

***N*-Cyclohexyl-1*H*-indole-2-carboxamide 4**
Elution with DCM-MeOH (0%-10%) yielded **4** as a white solid (yield 65%); mp 232-234 °C; IR (FTIR/FTNIR-ATR): 1616 cm^{-1} (C=O), 3237 cm^{-1} (N-H); ^{1}H-NMR ($CDCl_3$) δ: 9.41 (1H, s, indole H-1), 7.40 (1H, d, *J*=7.6 Hz, indole H-4), 7.39 (1H, d, *J*=8 Hz, indole H-7), 7.28 (1H, t, *J*=7.2 Hz, indole H-6), 7.13 (1H, t, *J*=7.2 Hz, indole H-5), 6.81 (1H, m, indole H-3), 6.04 (1H, d, J=7.6 Hz, NH), 4.04 (1H, m, cyclohexyl H-1), 2.08-1.18 (10H, m, cyclohexyl H-2,3,4,5,6); HRMS $C_{15}H_{19}N_2O$ $[M+H]^+$ *Calc*. 243.1497, Found m/z 243.1496.

***N*-Benzyl-1*H*-indole-2-carboxamide 5**
Elution with DCM-MeOH (0%-10%) yielded **5** as a white solid (yield 52%); mp 233.5-235 °C; IR (FTIR/FTNIR-ATR): 1628 cm^{-1} (C=O), 3259-3417 cm^{-1} (N-H). ^{1}H-NMR (DMSO-d_6) δ: 11.58 (1H, s, indole H-1), 9.01 (1H, t, *J*=6 Hz, NH), 7.58 (1H, d, *J*=8 Hz, indole H-4), 7.41 (1H, d, *J*=8.4 Hz, indole H-7), 7.32 (3H, m, indole H-3,5,6,), 7.24 (2H, m, benzyl H-2,6), 7.17 (2H, m, benzyl H-3,5), 7.00 (1H, m, benzyl H-4), 4.49 (2H, d, *J*=6 Hz, CH_2); HRMS $C_{16}H_{15}N_2O$ $[M+H]^+$ *Calc.* 251.1184, Found m/z 251.1191.

***N*-(4-Methoxybenzyl)-1*H*-indole-2-carboxamide 6**
Elution with DCM-MeOH (0%-10%) yielded **6** as a white solid (yield 55%); mp 226-228 °C; IR (FTIR/FTNIR-ATR): 1617 cm^{-1} (C=O), 3271-3412 cm^{-1} (N-H). ^{1}H-NMR ($CDCl_3$) δ: 9.42 (1H, s, indole H-1), 7.62 (1H, d, *J*=8 Hz, indole H-4), 7.43 (1H, d, *J*=8 Hz, indole H-7), 7.31-7.29 (2H, d, *J*=8.8 Hz, benzyl H-2,6), 7.15 (2H, m, indole H-5,6), 6.90 (2H, d, *J*=8.4 Hz, benzyl H-3,5), 6.82 (1H, s, indole H-3), 6.43 (1H, s, NH), 4.62 (2H, d, *J*=5.2 Hz, CH_2), 3.80 (3H, s, OCH_3). HRMS $C_{17}H_{17}N_2O_2$ $[M+H]^+$ *Calc.* 281.1290, Found m/z 281.1291.

BIOLOGICAL STUDIES

In vitro antioxidant activity

DPPH radical scavenging assay

Sweeping effects of synthesized ester and amid derivaties on DPPH free radicals were measured through 2,2-diphenyl-1-picrylhydrazyl stable radical's capability of removing the violet/purple color. DPPH assay was performed using test compounds as previously described (21). DPPH reacts with antioxidant compounds. It is reduced, and then its deep violet color in methanol bleached to yellow, showing a significant decrease in absorption at 517 nm.

According to the literature, DPPH ($4x10^{-4}$ M) was prepared fresh. Test samples (50-200 µg/ml) were dissolved in methanol and mixed with methanol solutions of DPPH in 96-well plates (each well contains 100 µl test sample and 100 µl DPPH solution). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. BHT (50-200 µg/ml) was used as a reference compound. Tests were carried out in triplicate (21). Free radical DPPH inhibition in percentage and was calculated using following equation. A_0 = the absorbance of the control; A_1 = the absorbance of the sample.

$$\text{Free radical DPPH inhibition (\%)} = [(A_0 - A_1 / A_0) \times 100]$$

Ferric ion (Fe^{2+}) chelating activity

Ferrous ion (Fe^{2+}) chelating activity was determined by inhibiting ferrous–ferrozine complex formation after treatment of test material with ferrous ion (Fe^{2+}).

Various concentrations of synthesized compounds **1-6** (50-200 µg/ml) in methanol were added to a solution of 2 mM $FeCl_2$ (10 µL) 96 well plate. The reaction was initiated by adding 5 mM ferrozine (3,2-(pyridyl)-5,6-bis(4-phenylsulfonicacid)-1,2,4-tirazine) (20 µL) to methanol. Then, the mixture was shaken vigorously at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. All experiments were performed in triplicate. The percentage ferrous ion chelating effects of the compounds (**1-6**) and standard compound EDTA (50-200 µg/ml) were

calculated using the following equation. AC is the absorbance of the control ($FeCl_2$ and ferrozine) and AS is the absorbance in the presence of the compounds (22).

Ferrous ions chelating effect (%) = [1 – (AS/AC)]x 100

Ferric ion (Fe^{3+}) reducing power

The reducing capacity for a compound can be measured by the direct reduction of $Fe[(CN)_6]^{3-}$ to $Fe[(CN)6]^{2-}$. Free Fe^{3+} addition to the reduced product leads to a formation of the intense Perl's Prussian blue complex ($Fe_4[Fe(CN)_6]_3$), which has strong absorbance at 700 nm. To 50 µl solution of various concentrations of synthesized compounds **1-6** in distilled water, 50 µl sodium phosphate buffer (0,2 M, pH 6.6) and 50 µl potassium ferricyanide ($K_3Fe(CN)_6$] (%1) were added, and the mixture was incubated at 50 °C for 20 minutes. After incubation period, the reaction mixture was acidified with 50 µl trichloroacetic acid (10%). After adding 10 µl $FeCl_3$, this solution, was incubated at 50 °C for another 10 minutes. The absorbance was measured at 700 nm in a spectrophotometer. The values were expressed as absorbance. High absorbance indicates high reducing power ability. BHT was used as reference compound. All experiments were performed in triplicate (23, 24).

Microobiology

Pseudomonas aeruginosa ATCC 27853 (American Type Culture Collection), *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 90018 were included in the study.

Standard powders of ciprofloxacin and fluconazole were obtained from the manufacturers. Stock solutions were dissolved in distilled water (ciprofloxacin and fluconazole). All bacterial isolates were subcultured in MHA plates and incubated overnight at 37 °C and all Candida isolates were subcultured in SDA plates at 35 °C for 24-48 h. The microorganisms were passaged at least twice to ensure purity and viability.

The solution of the new synthesized compounds and standard drugs were prepared at 512, 256, 128, 64, 32, 16, 8, 4, 2, 1 µg/mL concentrations, at 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.031, 0.015, 0.0075 µg/mL concentrations in the wells of microplates by diluting in MHB, respectively.

Bacterial susceptibility testing was performed according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) M100-S16 (25). The bacterial suspensions used for inoculation were prepared at 10^5cfu/mL by diluting fresh cultures at MacFarland 0.5 density (10^7cfu/mL). Suspensions of the bacteria at 10^5 cfu/ml concentration were inoculated to the twofold diluted solution of the compounds. There were 10^4 cfu/mL bacteria in the wells after inoculations. MHB was used for diluting the bacterial suspension and for twofold dilution of the compound. DMSO, pure microorganisms and pure media were used as control wells. A 10 µl bacteria inoculum was added to each well of the microdilution trays. The trays were incubated at 37 ^{0}C in a humid chamber and MIC endpoints were read after 24 h of incubation. All organisms were tested in triplicate in each run of experiments. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations. (MICs) were noted.

All Candida isolates were subcultured in SDA plates, and incubated at 35 ^{0}C for 24-48 h prior to antifungal susceptibility testing, and passaged at least twice to ensure purity and viability. Susceptibility testing was performed in RPMI-1640 medium with L-glutamine buffered pH 7 with MOPS and culture suspensions were prepared through the guideline of CLSI M27-A (26). The yeast suspensions used for inoculation were prepared at 10^4 cfu/ml by diluting fresh cultures at MacFarland 0.5 density (10^6 cfu/mL). Suspensions of the yeast at 10^4 cfu/mL concentration were inoculated to twofold diluted solution of the compounds. There were 10^3 cfu/mL bacteria in the wells after inoculations. A 10 µL yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35 ^{0}C in a humid chamber and MIC endpoints were read after 48 h of incubation. All organisms were tested in triplicate in each run of experiments. The lowest concentration of the compound that completely inhibits macroscopic

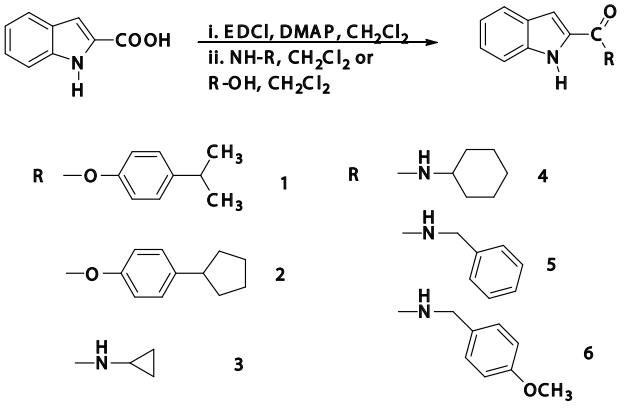
growth was determined and minimum inhibitory concentrations (MICs) were reported in Table 2.

RESULTS AND DISCUSSIONS

Chemistry

The desired ester and amide derivatives of indole-2-carboxylic acid were prepared in a one-pot synthetic procedure as shown in the Scheme. Treatment of indole-2-carboxylic acid with the appropriate phenol or amine derivatives in the presence of EDCI as the carboxyl group activator and DMAP afforded the desired products **1-6** in 37-65% yields. Chemical structures of these compounds were elucidated by their elemental analysis, IR, HRMS and ^{1}H-NMR spectral data. The IR spectra of 1*H*-indole-2-carboxylic acid ester and amide derivatives exhibited characteristic strong absorption bands for carbonyl group at 1614-1705 cm^{-1}, and N-H stretching bands at 3417-3237 cm^{-1}.

The ^{1}H-NMR spectra of compounds **1-6** displayed a singlet due to indole H-1 proton in the δ 11.58-9.06 ppm range showing the integration for one proton. In the ^{1}H-NMR spectra of compounds, signals of indole H-4 proton was observed as doublet in the δ 7.74-7.40 ppm range. In the ^{1}H-NMR spectra of amide derivatives **3-6**, signals of NH proton was detected in the δ 9.01-6.04 ppm range. Further spectroscopic details of these compounds are presented in the experimental part.



Scheme 1. Synthetic pathway for the synthesis of the target ester and amide derivatives.

PHARMACOLOGY

In vitro antioxidant activity

In the present study, the antioxidant and radical scavenging effects of the synthesized compounds (**1-6**) were determined in vitro with different bioanalytical methodologies. The antioxidant and radical scavenging activities of the compounds were compared with BHT, or EDTA according to test applied. These comparisons were performed using a series of in vitro tests including DPPH radical scavenging activity, reducing power (Fe^{3+}–Fe^{2+} transformation) and metal chelating on ferrous ion (Fe^{2+}) activities (Table 1).

The scavenging effects of synthesized compounds on DPPH radical were presented in Table 1. Preliminary screenings of title compounds were performed at different concentrations to determine scavenging effects on the DPPH. Although the scavenging rates were not very pronounced, the compounds **5** and **6** demonstrated scavenging effect in the range of 2-7%. The rest of the compounds had no effect on DPPH radical.

The reducing power associated with antioxidant activity reflects the electron donating capacity of bioactive compounds. As can be seen in Table 1, all the synthesized compounds (**1-6**) have powerful Fe^{3+} reducing abilities. Especially, compound **5** and **6** (*N*-benzyl and *N*-(4-methoxybenzyl)-1*H*-indole-2-carboxamide derivatives, respectively) showed excellent reducing power at 200, 150, 100 and 50 µg/ml concentrations as compared to BHT.

Ferrous ions (Fe^{2+} and Fe^{3+}) constitute the most effective pro-oxidants in food and biological systems. Removal of free iron ions from circulation is a promising approach to prevent oxidative stress-induced diseases. Ferrous chelation exhibits important antioxidative effects by delaying metal-catalyzed oxidation. Ferrous ion (Fe^{2+}) chelating activity was determined and results were shown in Table 1. All tested compounds **1-6** showed excellent metal chelating activity at 200, 150, 100 and 50 µg/ml concentrations as compared to EDTA. Especially, all of them had marked metal chelating activities 150, 100 and 50 µg/ml concentrations than EDTA which has not showed activity at those concentrations.

Table 1. Antioxidant properties of title compounds.

Compound	DPPH radical scavenging capacity (Inhibition %)	Chelating activity on metal ions %	Reducing power
1			
200 µg/ml	-	53.62±1.62	0.519±0.05
150 µg/ml	-	29.85±2.46	0.488±0.03
100 µg/ml	-	32.06±3.23	0.659±0.02
50 µg/ml	-	22.23±0.08	0.387±0.02
2			
200 µg/ml	-	57.16±0.90	0.218±0.01
150 µg/ml	-	19.29±2.99	0.178±0.01
100 µg/ml	-	16.98±0.72	0.150±0.02
50 µg/ml	-	-	0.105±0.01
3			
200 µg/ml	-	55.15±2.07	0.185±0.01
150 µg/ml	-	47.02±3.64	0.158±0.01
100 µg/ml	-	38.10±1.00	0.146±0.02
50 µg/ml	-	15.07±2.02	0.063±0.01
4			
200 µg/ml	-	58.72±3.99	0.226±0.02
150 µg/ml	-	29.00±3.37	0.217±0.01
100 µg/ml	-	5.28±1.18	0.302±0.07
50 µg/ml	-	-	0.091±0.01
5			
200 µg/ml	5.45±0.75	38.43±3.79	0.974±0.26
150 µg/ml	7.13±1.23	48.54±3.31	0.861±0.16
100 µg/ml	3.74±0.01	17.07±1.99	0.774±0.15
50 µg/ml	-	13.84±2.70	0.578±0.08
6			
200 µg/ml	7.07±0.70	56.91±4.59	1.073±0.08
150 µg/ml	5.70±0.18	50.53±5.09	0.947±0.09
100 µg/ml	3.16±0.40	51.07±1.12	0.951±0.01
50 µg/ml	2.94±0.97	9.73±2.77	0.932±0.03
BHT			
200 µg/ml	95.41±0.40	NT	0.998±0.10
150 µg/ml	95.23±0.08	NT	0.849±0.14
100 µg/ml	93.76±0.36	NT	0.679±0.08
50 µg/ml	80.08±0.62	NT	0.652±0.03
EDTA			
200 µg/ml	NT	6.24±0.19	NT
150 µg/ml	NT	-	NT
100 µg/ml	NT	-	NT
50 µg/ml	NT	-	NT

NT: Not tested, -: No Activity

Antimicrobial Activity

All the new synthesized derivatives were assayed *in vitro* for antimicrobial activity against two Gram-positive, two Gram-negative bacterial strains and two *Candida* strains. The MIC values were determined by a microdilution method in MHB and RPMI-1640 medium for the antibacterial and antifungal assays, respectively. According to the data (Table 2), all the compounds were inactive against *E.coli, P. aeruginosa* and *S. aureus*. Compound **2** was found the most active derivative against *E. faecalis*. Compound **2** also demonstrated significant activity against *C. albicans* with a MIC value of 8 µg/mL. On the other hand, compounds **1, 3, 5** and **6** had noticeable antifungal

Table 2. Antimicrobial activities of title compounds (µg/mL).

Compound	*E.coli*	*P. aeruginosa*	*S. aureus*	*E. faecalis*	*C. albicans*	*C. parapsilosis*
1	512	256	256	**128**	**32**	**64**
2	512	256	256	**64**	**8**	256
3	512	256	512	256	**64**	256
4	512	256	512	512	512	512
5	512	256	512	256	**64**	**128**
6	512	256	512	**128**	**32**	**128**
Ciprofloxacin	0.0075	0.25	0.125	0.5	-	-
Fluconazole	-	-	-	-	1	1

activities against *Candida* strains especially *C. albicans (MIC for **1** and **6**, 32; for **3** and **5**, 64* µg/mL). Compound **1** also demonstrated moderate activity against *C. parapsilosis* with a MIC value of 64 µg/mL.

CONCLUSION

The synthesis of ester and amide derivatives of indole-2-carboxylic acid was described along with their preliminary evaluation as potential antioxidant and antimicrobial agents. According to the results the synthesized compounds **1-6** had effective antioxidant power. All compounds showed excellent metal chelating activity as compared to EDTA. Compound **5** and **6** were exhibited very strong reducing power. Furthermore, antimicrobial evaluation of the presented structures against Gram-positive bacteria *E. faecalis* and fungi *C. albicans* are encouraging since compound **2** demonstrated potent antifungal properties. Further studies with these types of compounds are under ongoing investigation in our laboratory.

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