ORIGINAL ARTICLE

RUTIN SEMISYNTHETIC DERIVATIVES WITH ANTIFUNGAL PROPERTIES

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Abstract

Among the often cited flavonoids with pharmacological properties, rutin is recently highlighted for its antifungal effect. On the other hand, the development of azole based drugs represented a major advance in medical mycology; azoles are currently the most popular class of antifungals used in medicine (clotrimazole, ketoconazole, fluconazole, itraconazole, a.o.). Starting from these facts, we synthesised some water soluble rutin derivatives containing imidazole and benzimidazole moieties in their chemical structure, treating rutin with 1,3-dichloro-2-propanol, 1-bromo-3-chloropropane, 2-dibromoethane and dibromomethane, and then with imidazole and benzimidazole, respectively. Molecular formula, weight, yield, melting points and solubility have characterized the new derivatives. Elemental analysis, IR and ^{1}H-NMR spectral analysis have confirmed the structure of new the compounds. *In vitro* microbiological assays have been made. The tested compounds have shown a good antifungal activity (against *Candida spp*.) and antibacterial effects also (against Gram- positive bacterial strains).

Rezumat

Din categoria flavonoidelor cu proprietăţi farmacologice, rutozidul este recent investigat pentru proprietăţile sale antifungice. Pe de altă parte, dezvoltarea medicamentelor ce conţin azoli în structura chimică a constituit un progres major al terapiei antifungice, la ora actuală azolii reprezentând cea mai utilizată categorie de antimicotice (clotrimazol, ketoconazol, fluconazol, itraconazol, ş.a.). Pornind de la aceste premize, am sintetizat o serie de derivaţi hidrosolubili ai rutozidului, care conţin în structură fragmente de imidazol şi benzimidazol, prin tratarea rutozidului cu 1,3-dicloro-2-propanol, 1-brom-3-cloropropan, 2-dibromoetan şi dibromometan, în mediu de metoxid de sodiu, şi apoi cu imidazol, respectiv, benzimidazol. Noii derivaţi au fost caracterizaţi prin formulă şi masă moleculară, randament, punct de topire şi solubilitate, iar structura a fost confirmată prin analiză elementală şi analiză spectrală în IR şi ^{1}H-RMN. Au fost întreprinse teste microbiologice *in vitro*. Compuşii testaţi prezintă bune proprietăţi antifungice (împotriva *Candida spp.*), precum şi efecte antibacteriene (asupra bacteriilor gram-pozitive).

Keywords: flavonoids, rutin, imidazole, antifungal

Introduction

Several studies highlight antifungal properties of rutin or vegetal species with a high content of flavonoids (especially rutin); furthermore, there are some arguments that rutin is able to increase the anti-fungal activity of other compounds [2, 3, 5, 7, 8]. Unfortunately, rutin has a very low solubility in water, but our research team previously synthesized several rutin water-soluble derivatives [4]. On the other hand, it is well-known that azoles are important pharmacophores for antifungal effect, but also for antibacterial, anticancer, antiviral, and antiprotozoal actions. Imidazole ring is present in many antifungal existing drugs, like clotrimazole or miconazole. Besides, there are many recent studies concerning the design and synthesis of some new antifungal agents, incorporating azoles in their molecules [1, 6, 9, 10].

Starting from these premises, eight new rutin derivatives containing imidazole and benzimidazole moieties in their structures were synthesized and tested in antifungal and antibacterial assays.

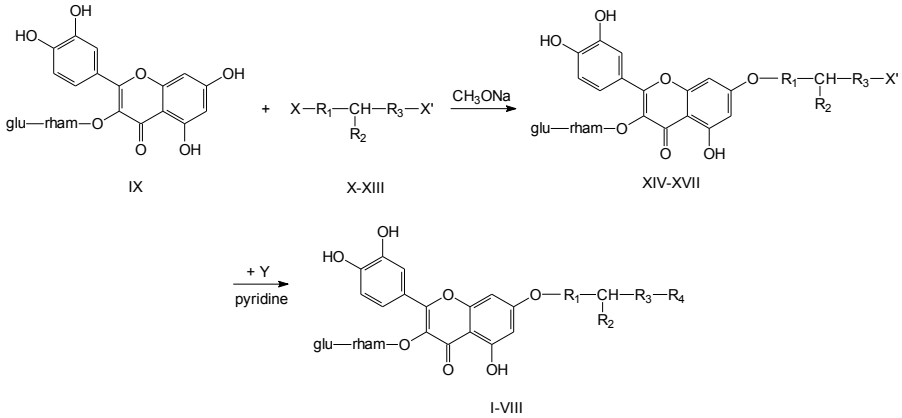
Materials and Methods

All commercial chemicals and solvents are reagent grade and were used without further purification. Rutin (97 - 102%) was purchased from Acros Organics, Belgium; solvents (methanol, ethanol, isopropanol,) were purchased from Sigma-Aldrich, Germany, halogenated reagents (1,3-dichloro-2-propanol, 1-brom-3-chloropropane, 1,2-dibromoethane and dibromomethane) were purchased from Merck-

Schuchardt, Germany, sodium was delivered by Riedel-de-Haen AG, Germany, and Silicagel was purchased from Fluka, Germany. Melting points are uncorrected and were measured in open capillary tubes on an Electrothermal Mel-Temp device; the elemental analysis was performed on an Exeter Analytical CE-440 elemental analyser. The IR spectra were recorded on a FT/IR Jasco 670 Plus spectrometer. The ^{1}H-NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer using tetramethylsilane as internal standard and DMSO-d6 as solvent. The antimicrobial activity was studied using Gram- positive bacteria (*Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341), Gram- negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and pathogenic yeasts (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC MYA 2950); all these strains were obtained from the Culture Collection of the Department of Microbiology, Faculty of Pharmacy, "Gr. T. Popa" University of Medicine and Pharmacy, Iaşi, Romania.

Rutin derivatives (**I - VIII**) were synthesized according to Figure 1; 6.64 g of rutin (**IX**) (0.01 mol) was solved in 180 mL sodium methoxide (containing 0.23 g sodium), reflux heating for 30 minutes, subsequently treating with 0.01 mol of 1,3-dichloro-2-propanol; 1-brom-3-chloropropane; 1,2-dibromoethane and dibromomethane, respectively (**X - XIII**) and finally, reflux heating for six hours with imidazole and benzimidazole, respectively, in the presence of pyridine, afforded the corresponding compounds **I - VIII**. From reaction mixtures, which are yellow-orange solutions, we obtained the crude derivatives through precipitate with isopropanol, filtration and ambient temperature drying.

The crude compounds were purified by column chromatography (0.5 cm x 15 cm, Silicagel H (10 - 40 μm), elution with 50° alcohol: 0.25 - 0.30 mL/min).



Comp. no.	X	X'	R_1	R_2	R_3	Y	R_4
I	Cl	Cl	CH_2	OH	CH_2		
II	Br	Cl	CH_2	H	CH_2		
III	Br	Br	CH_2	H	-		
IV	Br	Br	-	H	-		
V	Cl	Cl	CH_2	OH	CH_2		
VI	Br	Cl	CH_2	H	CH_2		
VII	Br	Br	CH_2	H	-		
VIII	Br	Br	-	H	-		

Figure 1.
The synthesis of rutin derivatives **I - VIII**

Antimicrobial tests of selected microorganisms were carried out using a disc-diffusion method [11, 12]. A small amount of each microbial culture was diluted in sterile 0.9% NaCl until the turbidity was equivalent to McFarland standard no. 0.5 (106 CFU/mL). The suspensions were further diluted 1:10 in Müeller Hinton agar for bacteria (Oxoid) and Müeller-Hinton agar for yeasts (HiMedia) and then spread on sterile Petri plates (25 mL/Petri plate). Sterile stainless steel cylinders (5 mm internal diameter; 10 mm height) were applied on the agar surface in Petri plates. Then, 0.1 mL of each compounds (**I - VIII**) were added into cylinders. Commercial available discs containing Ciprofloxacin (5 μg/disc), Fluconazole (25 μg/disc), Voriconazole (1 μg/disc) and Nystatin (100 μg/disc) were used as positive controls. The plates were incubated at 37°C for 24 h (bacteria) and at 24°C for 48 h (yeasts). After incubation, the diameters of inhibition zones were measured in mm, including disc size.

Results and Discussion

Eight new derivatives of rutin were synthesised and purified by column chromatography in good yield (72% - 86%); all these compounds are crystalline, hygroscopic, yellow powders, with no odour and having a slightly bitter taste, soluble in water,

alcohol, dimethylsulfoxide and dimethylformamide and insoluble in 2-propanol, dioxane, acetone, ether, benzene and chloroform. The chemical structures were proved by C, H, N elemental analysis and by IR and ^{1}H NMR spectroscopy.

3-[[6-O-(6-deoxy-α-L-manopyranosyl)-β-D-gluco-pyranosyl]-oxy]-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(oxy-1-(β-hydroxy-propyl)-3-(imidazol-1-yl))-4H-1-benzopyran-4-one (**I**): yield 86%; mp 243 - 246°C; IR (KBr) (cm^{-1}): 3400 (OH), 2920 (CH), 1700 (C=N), 1660 (C=O on aromatic ring), 1630 (C=C on imidazole ring), 1600 (aromatic structure), 1500 (aromatic C=C), 1360, 1300, 1200, 1070 (C-O-C), 1220 (C-N), 780 (aromatic substituents); ^{1}H NMR (DMSO-d6): δ ppm: 7.74 (d, 1H, H-6'), 7.60 (d, 1H, N=CH-N), 7.44 (d, 1H, H-2'), 7.20 (d, 1H, N-CH=C), 6.90 (d, 1H, CH imidazole), 6.73 (d, 1H, H-5'), 6.46 (s, 1H, H-8), 6,31 (s, 1H, H-6), 5.52 (s, 1H, H1-glucosyl), 4.46 (s, 1H, H1-rhamnosyl), 4.32 (s, 2H, O^7-CH_2), 4.1 (d, 1H, CH alkyl chain), 3.90 (s, 2H, -CH_2-N), 3.60, 3.20 (d, 4H, CH glu), 3.10, 3.03 (d, 4H, CH rha), 1.27 (s, 3H, CH_3 rha); Molecular formula: $C_{33}H_{38}N_2O_{17}$; Molecular weight = 734.66; Calculated = C (53.95%) H (5.21%) N (3.81%); Found = C (53.92%) H (5.20%) N (3.79%).

3-[[6-O-(6-deoxy-α-L-manopyranosyl)-β-D-gluco-pyranosyl]-oxy]-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(oxy-1-propyl-3-(imidazol-1-yl))-4H-1-benzopyran-4-one (**II**): yield 75.5%; mp 212 - 214°C; IR (KBr) (cm^{-1}): 3380 (OH), 2910 (CH), 1700 (C=N), 1650 (C=O on aromatic ring), 1630 (C=C on imidazole ring), 1600 (aromatic structure), 1520 (aromatic C=C), 1360, 1310, 1220, 1090 (C-O-C), 1230 (C-N), 790 (aromatic substituents); ^{1}H NMR (DMSO-d6): δ ppm: 7.74 (d, 1H, H-6'), 7.57 (d, 1H, N=CH-N), 7.44 (d, 1H, H-2'), 7.18 (d, 1H, N-CH=C), 6.85 (d, 1H, CH imidazole), 6.74 (d, 1H, H-5'), 6.46 (s, 1H, H-8), 6,30 (s, 1H, H-6), 5.53 (s, 1H, H1-glucosyl), 4.46 (s, 1H, H1-rhamnosyl), 4.23 (s, 2H, O^7-CH_2), 3.78 (d, 2H, -CH_2-N), 3.60, 3.20 (d, 4H, CH glu), 3.14, 3.02 (d, 4H, CH rha), 2.14 (m, 2H, C-CH_2-C), 1.27 (s, 3H, CH_3 rha). Molecular formula: $C_{33}H_{38}N_2O_{16}$; Molecular weight = 718.66; Calculated = C (55.15%) H (5.32%) N (3.89%); Found = C (55.09%) H (5.30%) N (3.85%).

3-[[6-O-(6-deoxy-α-L-manopyranosyl)-β-D-gluco-pyranosyl]-oxy]-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(oxy-1-ethyl-2-(imidazol-1-yl))-4H-1-benzopyran-4-one (**III**): yield 82%; mp 222 - 224°C; IR (KBr) (cm^{-1}): 3370 (OH), 2900 (CH), 1710 (C=N), 1655 (C=O on aromatic ring), 1640 (C=C on imidazole ring), 1600 (aromatic structure), 1510 (aromatic C=C), 1350, 1300, 1225, 1080 (C-O-C), 1220 (C-N), 800 (aromatic substituents); ^{1}H NMR (DMSO-d6): δ ppm: 7.73 (d, 1H, H-6'), 7.59 (d, 1H, N=CH-N), 7.44 (d, 1H, H-2'), 7.19 (d, 1H, N-CH=C), 6.88 (d, 1H, CH imidazole), 6.73 (d, 1H, H-5'), 6.46 (s, 1H, H-8), 6,30 (s, 1H, H-6), 5.53 (s, 1H, H1-glucosyl), 4.49 (d, 2H, O^7-CH_2), 4.46 (s, 1H, H1-rhamnosyl), 4.10 (d, 2H, -CH_2-N), 3.60, 3.20 (d, 4H, CH glu), 3.14, 3.02 (d, 4H, CH rha), 1.27 (s, 3H, CH_3 rha); Molecular formula: $C_{32}H_{36}N_2O_{16}$; Molecular weight = 704.64; Calculated = C (54.54%) H (5.14%) N (3.97%); Found = C (54.58%) H (5.16%) N (3.96%).

3-[[6-O-(6-deoxy-α-L-manopyranosyl)-β-D-gluco-pyranosyl]-oxy]-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(oxy-methyl-imidazol-1-yl))-4H-1-benzopyran-4-one (**IV**): yield 72%; mp 230 - 234°C; IR (KBr) (cm^{-1}): 3390 (OH), 2912 (CH), 1715 (C=N), 1670 (C=O on aromatic ring), 1642 (C=C on imidazole ring), 1604 (aromatic structure), 1510 (aromatic C=C), 1355, 1300, 1220, 1075 (C-O-C), 1210 (C-N), 810 (aromatic substituents); ^{1}H NMR (DMSO-d6): δ ppm: 7.73 (d, 1H, H-6'), 7.50 (d, 1H, N=CH-N), 7.44 (d, 1H, H-2'), 7.28 (d, 1H, N-CH=C), 6.87 (d, 1H, CH imidazole), 6.73 (d, 1H, H-5'), 6.45 (s, 1H, H-8), 6,42 (s, 1H, H-6), 5.63 (d, 2H, O^7-CH_2-N), 5.53 (s, 1H, H1-glucosyl), 4.46 (s, 1H, H1-rhamnosyl), 3.60, 3.20 (d, 4H, CH glu), 3.14, 3.02 (d, 4H, CH rha), 1.27 (s, 3H, CH_3 rha); Molecular formula: $C_{31}H_{34}N_2O_{16}$; Molecular weight = 690.61; Calculated = C (53.91%) H (4.96%) N (4.05%); Found = C (53.85%) H (5.00%) N (4.00%).

3-[[6-O-(6-deoxy-α-L-manopyranosyl)-β-D-gluco-pyranosyl]-oxy]-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(oxy-1-(β-hydroxy-propyl)-3-(benzimidazol-1-yl))-4H-1-benzopyran-4-one (**V**): yield 78.4%; mp 271 - 273°C; IR (KBr) (cm^{-1}): 3390 (OH), 3240 (CH heteroaromatic ring in benzimidazole), 2924 (CH), 1700 (C=N), 1660 (C=O on aromatic ring), 1630 (C=C on benzimidazole ring), 1600 (aromatic structure), 1500 (aromatic C=C), 1360, 1310, 1205, 1080 (C-O-C), 804 (aromatic substituents); ^{1}H NMR (DMSO-d6): δ ppm: 8.04 (d, 1H, N=CH-N), 7.73 (d, 1H, H-6') , 7.65, 7.00 (m, 4H, Ar-H), 7.44 (d, 1H, H-2'), 6.74 (d, 1H, H-5'), 6.46 (s, 1H, H-8), 6,31 (s, 1H, H-6), 5.52 (s, 1H, H1-glucosyl), 4.46 (s, 1H, H1-rhamnosyl), 4.38 (s, 2H, -CH_2-N), 4.30 (s, 2H, O^7-CH_2), 4.28 (m, 1H, CH alkyl chain), 3.60, 3.20 (d, 4H, CH glu), 3.10, 3.03 (d, 4H, CH rha), 1.27 (s, 3H, CH_3 rha); Molecular formula: $C_{37}H_{40}N_2O_{17}$; Molecular weight = 784.72; Calculated = C (56.63%) H (5.13%) N (3.57%); Found = C (56.55%) H (5.15%) N (3.55%).

3-[[6-O-(6-deoxy-α-L-manopyranosyl)-β-D-gluco-pyranosyl]-oxy]-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(oxy-1-propyl-3-(benzimidazol-1-yl))-4H-1-benzo-pyran-4-one (**VI**): yield 84.5%; mp 280 - 282°C; IR (KBr) (cm^{-1}): 3370 (OH), 3210 (CH heteroaromatic ring in benzimidazole), 2930 (CH), 1680 (C=N), 1650 (C=O on aromatic ring), 1625 (C=C on benz-imidazole ring), 1590 (aromatic structure), 1510

(aromatic C=C), 1340, 1300, 1214, 1070 (C-O-C), 800 (aromatic substituents); ^{1}H NMR (DMSO-d6): δ ppm: 8.00 (d, 1H, N=CH-N), 7.73 (d, 1H, H-6') , 7.65, 7.00 (m, 4H, Ar-H), 7.44 (d, 1H, H-2'), 6.74 (d, 1H, H-5'), 6.46 (s, 1H, H-8), 6,31 (s, 1H, H-6), 5.52 (s, 1H, H1-glucosyl), 4.46 (s, 1H, H1-rhamnosyl), 4.25 (s, 2H, O^7-CH_2), 4.14 (s, 2H, -CH_2-N), 3.60, 3.20 (d, 4H, CH glu), 3.10, 3.03 (d, 4H, CH rha), 2.23 (m, 2H, C-CH_2-C), 1.27 (s, 3H, CH_3 rha); Molecular formula: $C_{37}H_{40}N_2O_{16}$; Molecular weight = 768.72; Calculated = C (57.81%) H (5.24%) N (3.64%); Found = C (57.79%) H (5.22%) N (3.63%).

3-[[6-O-(6-deoxy-α-L-manopyranosyl)-β-D-glucopyranosyl]-oxy]-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(oxy-1-ethyl-2-(benzimidazol-1-yl))-4H-1-benzopyran-4-one (**VII**): yield 75.5%; mp 261 - 262°C; IR (KBr) (cm^{-1}): 3340 (OH), 3234 (CH heteroaromatic ring in benzimidazole), 2900 (CH), 1682 (C=N), 1640 (C=O on aromatic ring), 1620 (C=C on benzimidazole ring), 1600 (aromatic structure), 1500 (aromatic C=C), 1320, 1290, 1200, 1050 (C-O-C), 820 (aromatic substituents); ^{1}H NMR (DMSO-d6): δ ppm: 8.03 (d, 1H, N=CH-N), 7.73 (d, 1H, H-6') , 7.66, 7.00 (m, 4H, Ar-H), 7.44 (d, 1H, H-2'), 6.74 (d, 1H, H-5'), 6.46 (s, 1H, H-8), 6,31 (s, 1H, H-6), 5.53 (s, 1H, H1-glucosyl), 4.46 (s, 1H, H1-rhamnosyl), 4.39 (d, 2H, -CH_2-N), 4.31 (s, 2H, O^7-CH_2-), 3.60, 3.20 (d, 4H, CH glu), 3.10, 3.03 (d, 4H, CH rha), 1.27 (s, 3H, CH_3 rha); Molecular formula: $C_{36}H_{38}N_2O_{16}$; Molecular weight = 754.70; Calculated = C (57.29%) H (5.07%) N (3.71%); Found = C (57.27%) H (5.04%) N (3.70%).

3-[[6-O-(6-deoxy-α-L-manopyranosyl)-β-D-glucopyranosyl]-oxy]-2-(3,4-dihydroxyphenyl)-5-hydroxy-7-(oxy-methyl-imidazol-1-yl))-4H-1-benzopyran-4-one (**VIII**): yield 85%; mp 286 - 288°C; IR (KBr) (cm^{-1}): 3330 (OH), 3230 (CH heteroaromatic ring in benzimidazole), 2910 (CH), 1680 (C=N), 1634 (C=O on aromatic ring), 1610 (C=C on benzimidazole ring), 1580 (aromatic structure), 1500 (aromatic C=C), 1310, 1294, 1210, 1020 (C-O-C), 800 (aromatic substituents); ^{1}H NMR (DMSO-d6): δ ppm: 8.07 (d, 1H, N=CH-N), 7.73 (d, 1H, H-6') , 7.69, 7.00 (m, 4H, Ar-H), 7.44 (d, 1H, H-2'), 6.74 (d, 1H, H-5'), 6.40 (s, 1H, H-8), 6,37 (s, 1H, H-6), 5.74 (d, 2H, O^7-CH_2-N), 5.53 (s, 1H, H1-glucosyl), 4.46 (s, 1H, H1-rhamnosyl), 3.60, 3.20 (d, 4H, CH glu), 3.10, 3.03 (d, 4H, CH rha), 1.27 (s, 3H, CH_3 rha); Molecular formula: $C_{35}H_{36}N_2O_{16}$; Molecular weight = 740.67; Calculated = C (56.75%) H (4.89%) N (3.78%); Found = C (56.72%) H (4.92%) N (3.77%).

The diameters of the inhibition zones (in mm) corresponding to the tested substances are shown in Table I. All assays were carried out in triplicate. Results are expressed as means ± SD.

Table I

Antimicrobial activity of rutin derivatives **I - VIII**

Compounds	Diameter of inhibition zones (mm)					
	S. aureus ATCC 25923	*S. lutea* ATCC 9341	*E. coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*C. albicans* ATCC 90028	*C. glabrata* ATCC MYA 2950
I	**13.3** ± 0.57	**20.0** ± 0.00	0	0	**10.3** ± 0.57	**11.0** ± 0.00
II	**12.6** ± 0.57	**20.3** ± 0.57	0	0	**10.0** ± 0.00	**10.0** ± 0.00
III	**12.0** ± 0.00	**20.0** ± 0.00	0	0	**12.0** ± 0.00	**11.0** ± 0.00
IV	**13.3** ± 0.57	**20.0** ± 0.00	0	0	**10.3** ± 0.57	**11.0** ± 0.00
V	**13.0** ± 0.00	**18.0** ± 0.00	0	0	**10.0** ± 0.00	**12.3** ± 0.57
VI	**13.3** ± 0.57	**20.0** ± 0.00	0	0	**12.7** ± 0.06	**10.3** ± 0.57
VII	**13.0** ± 0.00	**19.3** ± 0.57	0	0	**10.3** ± 0.57	**11.0** ± 0.00
VIII	**13.0** ± 0.00	**20.0** ± 0.00	0	0	**10.3** ± 0.57	**13.3** ± 0.57
Ciprofloxacin (5 µg/disc)	24.7 ± 0.06	30.0 ± 0.00	30.5 ± 0.50	30.0 ± 0.00	*NT	*NT
Fluconazole (25 µg/disc)	NT*	NT*	NT*	NT*	30.3 ± 0.57	23.5 ± 0.50
Voriconazole (1 µg/disc)						
Nystatin (100 µg/disc)	NT*	NT*	NT*	NT*	23.3 ± 0.57	22.0 ± 0.00

*NT - not tested

The *in vitro* antimicrobial activity of rutin derivatives **I - VIII** were investigated against Gram- positive bacteria (*Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and pathogenic yeasts (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC MYA 2950) and were compared with commercial discs containing Ciprofloxacin (5 µg/disc), Fluconazole (25 µg/disc), Voriconazole (1 µg/disc) and Nystatin (100 µg/disc). The tested compounds showed good antibacterial activity on Gram- positive tested species and against all of the tested *Candida* spp. strains (Table I).

We have not registered antibacterial effect against Gram- negative species.

Conclusions

Eight new water-soluble rutin derivatives containing imidazole and benzimidazole moieties in their structures were synthesised; molecular formula, weight, yield, melting points and solubility have characterized the new derivatives. Elemental analysis and spectral analysis in IR and H-NMR have confirmed the structure of new compounds. The tested compounds have shown a good activity against *Candida* spp. and against Gram- positive bacterial strains and no activity against Gram-negative tested strains.

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