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Article · June 2020

DOI: 10.33263/BRIAC106.73297336

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Article
Volume 10, Issue 6, 2020, 7329 - 7336
https://doi.org/10.33263/BRIAC106.73297336

Investigation of the Antioxidant and Antibacterial Activity of Novel Quercetin Derivatives

Shaker A. N. Aljadaan [1], Rita S Elias [2,*], Reham Adnan Al-Anssari [3,*]

[1] Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Basrah, Iraq
[2] Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Basrah, Iraq
[3] Department of pharmacognosy and medicinal plants, College of Pharmacy, University of Basrah, Basrah, Iraq
* Correspondence: rita_phar@yahoo.com;

Scopus Author ID 35083419300
Received: 28.04.2020; Revised: 1.06.2020; Accepted: 3.06.2020; Published: 8.06.2020

Abstract: Our previous work involved the preparation and characterization of six quercetin derivatives, three of which were novel Schiff bases, while the fourth was a novel ionic salt of iodine. This study involved the investigation of the *in vitro* antioxidant activity against DPPH free radical as well as the *in vitro* antibacterial activity against *Bacillus cereus*, *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* of quercetin and its 1-6 derivatives. The results revealed that all the studied quercetin derivatives had shown less antioxidant and antibacterial activity than quercetin itself, except that of compounds 3 and 4, which displayed an improvement in the antibacterial activity against *Escherichia coli* as compared to that of quercetin.

Keywords: Quercetin; antioxidant; DPPH; antibacterial.

1. Introduction

Quercetin (Figure 1) is a pentahydroxyflavone that is considered to be one of the most important flavonoids because of its widespread within the food, broad biological actions and versatile physiological effects such as antioxidant, anticancer, antimicrobial, anti-inflammatory, anti-diabetic, prevention of cardiovascular disease, neuroprotective, and anti-obesity activities [1-18].

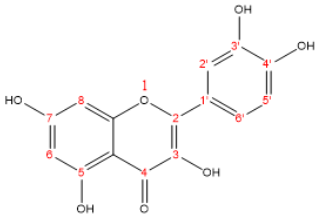


Figure 1. Chemical structure of quercetin.

Schiff bases, on the other hand, involve compounds that bear an imine or azomethine group within their chemical structure [19, 20] (Figure 2) such group has been linked to the biological activity of many compounds, such as antimalarial, antibacterial, antifungal, and antiviral activities [20-22].

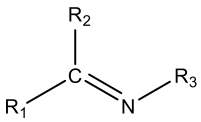


Figure 2. Chemical structure of Schiff base.

The antioxidant activity has been chosen to be investigated due to the tight relationship between oxidative stress and several malignant diseases such as cancer and cardiovascular pathologies, which point to the important role of the antioxidant compounds in maintaining the integrity of our living systems. Our study also involves an *in vitro* study of the potential antibacterial activity of quercetin and its (**1-6**) analog due to the spreading out of bacterial resistance to most known antibiotics and hence increasing the requirement to develop new antibacterial agents.

2. Materials and Methods

2.1. Materials.

DPPH, DMSO, ethanol 99% were purchased from Sigma-Aldrich (Germany), while Molar Hinton agar, Mannitol salt agar were obtained from Himedia (India), and EMB agar from oxoid (United Kingdom).

2.2. Chemical synthesis.

Six quercetin derivatives (compound 1-6) were synthesized and described previously [23] (Figure 3).

2.3. In vitro Antioxidant activity of quercetin and its 1-6 derivatives.

Studying the *in vitro* antioxidant activity of the quercetin and its 1-6 derivatives was performed by monitoring their effect on the absorbance of 0.1 mM of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) at 517mM following the procedure described by U. Krings and R.G. Berger [24] and Zheng Chen and coworkers [25]. 1mM ethanolic stock solutions of quercetin and the studied derivatives were prepared, from which different concentrations (0.1, 0.05, 0.02, 0.01, 0.001 mM) were made by mixing appropriate volumes with ethanol and 0.1mL of 1mM ethanolic DPPH solution followed by incubation in the darkness for about 30 minutes. The absorbance of each concentration was then measured at 517 nm using a quartz cell. Each concentration was established in triplicate and the percentages of inhibitory activity to DPPH of each concentration after 30 minutes was calculated from the equation (Eq.1) and were plot against tested concentrations in order to determine the amount required to reduce the initial concentration of DPPH by 50% (IC_{50}) for each compound.

$$\%\ DPPH_{remaining} = (A_{control} - A_{sample})/A_{control} * 100\% \quad (Eq.1)$$

Where $\%\ DPPH_{remaining}$ represents the percentage of the remaining DPPH after 30 minutes of sample addition; A_{sample} is the absorbance of the DPPH radical in the presence of sample; while $A_{control}$ refers to the absorbance of the 0.1mM DPPH radical alone.

2.4. In vitro Antibacterial activity of quercetin and its 1-6 derivatives.

Agar well diffusion method was used to explore the antibacterial activity of quercetin and (1-6) derivatives as described by Mounyer Balouiri and coworkers [26]. The antibacterial activity was studied against 4 types of bacteria namely *Bacillus cereus*, *Escherchia coli*, *Salmonella* spp and *Staphylococcus aureus* Three concentrations of each compound were prepared (0.25, 0.5 and 1 mg/mL) by dissolving quercetin and compounds 1, 2, 5 and 6 in DMSO while compounds 3 and 4 were decomposed in this solvent so were dissolved in absolute ethanol. A day before, each bacterial strain was activated by spreading them on their

selective media and keeping them for 24 hours within incubator (Binder, FD23, USA), then a diluted suspension of each bacteria was prepared using 0.9% NaCl. A suitable concentration was determined by comparing bacterial suspension turbidity against 0.5 McFarland standard. Mueller – Hinton agar plate surface was inoculated by the spreading of the bacterial inoculum over the whole agar surface using swap. Then, a hole with a diameter of 5 mm was made with a sterile yellow tip, and a 100 µL of each of the desired concentration of the studied compounds were injected into the well. A control well within the same agar plate was injected with DMSO or ethanol. The agar plates were then incubated for 18-24 hours at 37°C in the incubator, after which the diameter of the inhibition zone was measured. The introduced compounds were diffused in the agar medium and act to inhibit the growth of the tested bacterial strain. Selected antibiotic discs were introduced into other agar plates inoculated with similar bacterial strains to test bacterial sensitivity to them.

2.5. Statical analysis.

Data were analyzed with Microsoft excel 2010, and results were expressed as mean ± standard deviation. All measurements were evaluated using a student t-test designed to examine the difference in activity between the standard quercetin and its 1-6 derivatives. A *p*-value <0.05 was used to assess significance.

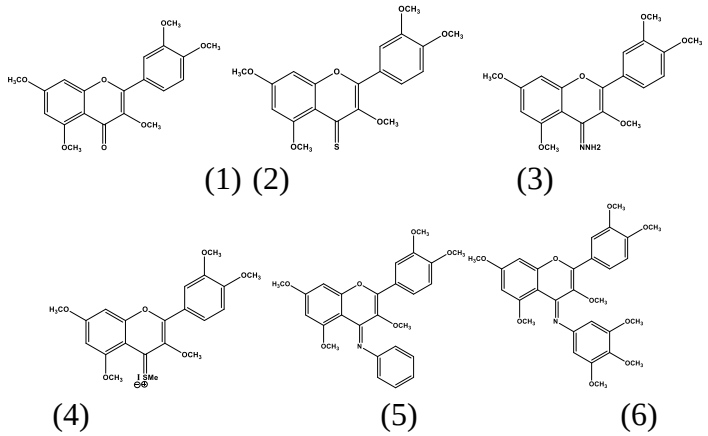


Figure 3. Chemical structure of compounds 1-6.

3. Results and Discussion

3.1. In vitro Antioxidant activity of quercetin and its 1-6 derivatives.

In vitro antioxidant activity of quercetin and its 1-6 derivatives study results are shown in Table 1 and figures 4 and 5. The radical scavenging activity of the prepared quercetin derivatives was estimated by monitoring the reduction of light absorbance at 517 nm of DPPH radical upon its reaction with different concentrations of quercetin derivatives in ethanol, then their percentages of antiradical activity were calculated as shown in equation (Eq.2) and plotted against the different studied concentrations in order to get the equation necessary to verify the half-maximal inhibitory concentration (IC_{50}) for each studied compound against DPPH as shown in Table 1 and Figures 4 and 5.

$$\% \text{ Of antioxidant activity} = (A - A_{30min.})/A * 100\% \qquad \text{(Eq.2)}$$

Where A account for the absorption of DPPH solution, A_{30} absorption of DPPH after 30 minutes of antioxidant addition.

Quercetin molecule is characterized by high antioxidant activity that is superior to vitamin C when both were used at low concentrations [27], this activity referred to the strong electron-donating effect of the hydroxyl groups to the conjugated π-system and the relative planer structure of the quercetin molecule which aid in charge distribution over the whole molecule. Therefore it is expected from the studied compounds (1-6) to show lower antioxidant activity than the parent compound since all the hydroxyl groups within the quercetin molecule were methylated and as were confirmed by previous studies [6, 28]. However, the IC_{50} values of the studied compounds 3 and 4 revealed the highest antioxidant activity, followed by compound 6, 2,5, and 1 subsequently. In fact, at 0.1mM concentration compound, 4 showed comparable radical scavenging activity to quercetin, but unlike quercetin [27] this activity rapidly dropped at a lower concentration, whereas a slower decline in the antioxidant potential was exposed by compound 3 at similar concentrations, giving it better IC_{50} values than compound 4.

The antioxidant activity of compound 4 might be referred to the presence of iodine in the ionic form within compound 4 chemical structure, iodine is known for its antiradical effects, iodide ion (I^-) react with free radicals (R) to give iodine radical ($I^\bullet$) which immediately converted into molecular iodine, the later is considered to be a good oxidant and potential pro-oxidant agent as shown in equations (Eq.3) and (Eq.4)[29].

$$R^\cdot + I^- \rightarrow R: + I \qquad \text{(Eq.3)}$$

$$2I^\cdot \rightarrow I_2 \qquad \text{(Eq.4)}$$

In addition to the direct interaction with free radicals of iodine, some studies have correlated it's *in vivo* antioxidant activity with its ability to iodinate certain amino acids and fatty acids and thus reducing their ability to oxidize themselves [29, 30]. On the other hand, the scavenging activity of compound 3 can be attributed to the presence of the NH_2 group in the hydrazone moiety, which can donate a hydrogen atom to the DPPH free radical, and the resulting free radical would be possibly stabilized by delocalization into the flavone backbone. On the other hand, the antioxidant activity of compounds 5, 2, 6 and 1 are close to each other and much lower than the previously mentioned compounds, possibly because there is no free available hydrogen atom to be donated directly to stabilize the DPPH radical.

3.2. In vitro Antibacterial activity of quercetin and its 1-6 derivatives.

The antibacterial activities of quercetin and its 1-6 derivatives were assayed *in vitro* against four types of bacteria, two of these were gram-positive (*Bacillus cereus* and *Staphylococcus aureus*), and two were gram-negative bacteria (*Escherichia coli* and *Salmonella* spp.). At tested concentrations, a variable degree of antibacterial activity have been detected among the studied compounds against *Staphylococcus aureus*, while *Escherichia coli* growth inhibition was detected only for compounds 3 and 4, and neither quercetin nor any of the studied derivatives have shown any activity against *Bacillus cereus* or *Salmonella* spp. Additionally, compounds 1, 2, 5, and 6 demonstrate no antibacterial activity at studied concentrations, possibly due to an increase in the hydrophobicity of the molecule [28, 31]. Antibacterial activity results of quercetin and compounds 1-6 were displayed in Table 2 and figure 6-8. Two standard antibiotic disks were used for the comparison and found to exert their antibacterial action at relatively lower concentrations than our studied compounds.

Table 1. The percentage of activity of different concentrations of quercetin and compounds 1-6 against DPPH.

Concentration (mM)	Compounds Activity (%)						
	Quercetin	**1[a]**	**2[a]**	**3**	**4**	**5[a]**	**6[a]**
0.1	93.3±0.37	9.6±5.1	13.3±4.5	82.4±0.5	92.9+1.0	11.5±1.2	23.0±1.6
0.05	92.1±0.25	6.3±0.9	13.2±3.2	68.5±4.0	55.3±6.2	4.6±0.6	14.5±3.3
0.02	84.2±0.21	3.5±2.3	4.5±0.5	37.5±6.5	19.5±2.6	2.6±0.4	8.7±1.7
0.01	61.9±0.78	3.0±0.4	2.2±1.4	19.3±5.3	5.3±1.6	2.3±1.4	1.3±1.0
0.001	20.3±0.46	2.1±2.4	0.93±1.1	2.7±2.9	0.71±0.7	1.4±1.6	3.7±0.8
IC_{50}	0.004	0.62	0.36	0.04	0.05	0.49	0.22

[a] have statically different antioxidant activity compared to that of quercetin

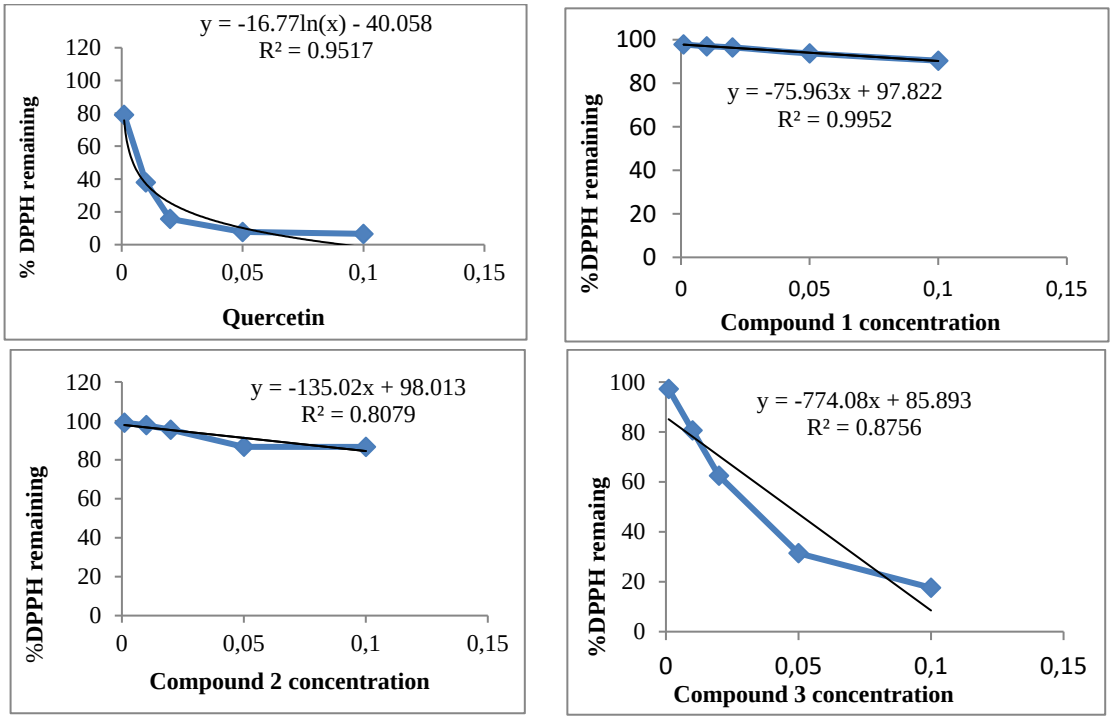


Figure 4. The effect of different concentrations of quercetin and compounds 1-3 on the percentage of DPPH remaining.

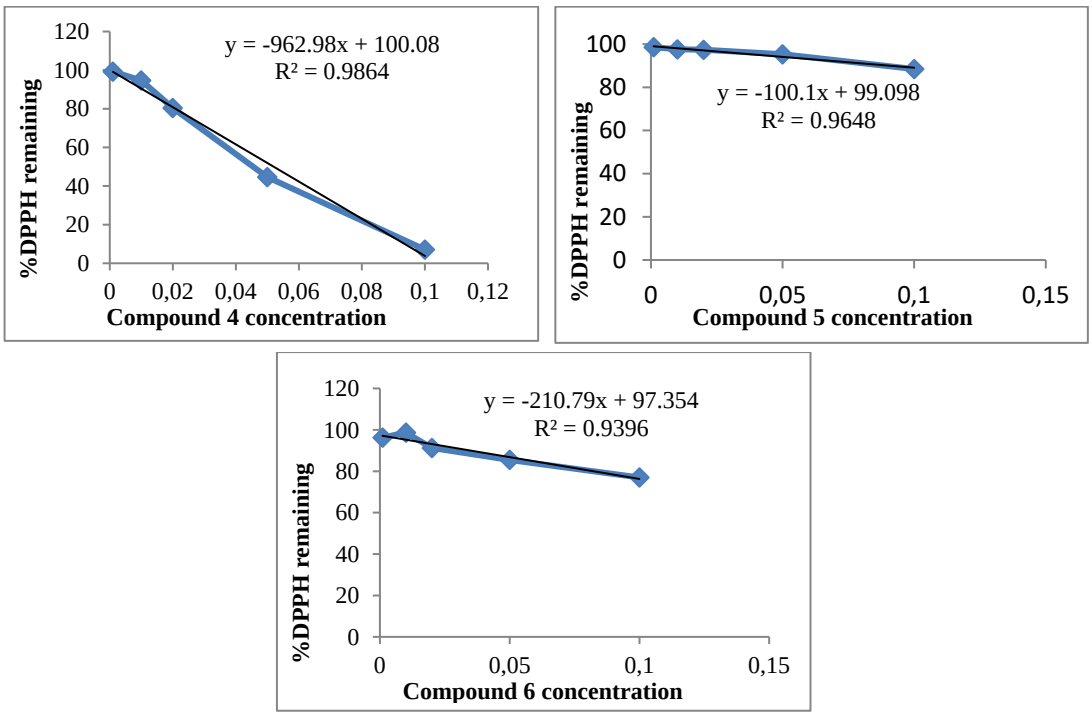


Figure 5. The effect of different concentrations compounds 4-6 on the percentage of DPPH remaining.

Table 2. Antibacterial activity of quercetin and compounds 1-6 against bacterial strains.

Compound	Conc. mg/mL	Mean Inhibition zone diameter (mm)a			
		Staph. aureus	***B. cereus***	***E. coli***	***Salmonella* spp.**
Quercetin	0.25				
	0.5	7			
	1	12			
1	0.25				
	0.5				
	1				
2	0.25				
	0.5				
	1				
3	0.25	6		6	
	0.5	7		6	
	1	12		7	
4	0.25			6	
	0.5	6		7	
	1	8		11	
5	0.25				
	0.5				
	1				
6	0.25				
	0.5				
	1				
Ciprofloxacin	0.01	31	28	32	20
Gentamicin	0.01	19	20	15	15

[a] Hole diameter (= 5mm) was not subtracted.

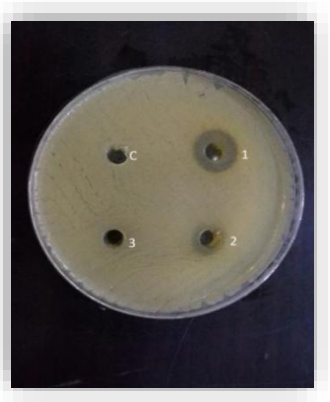
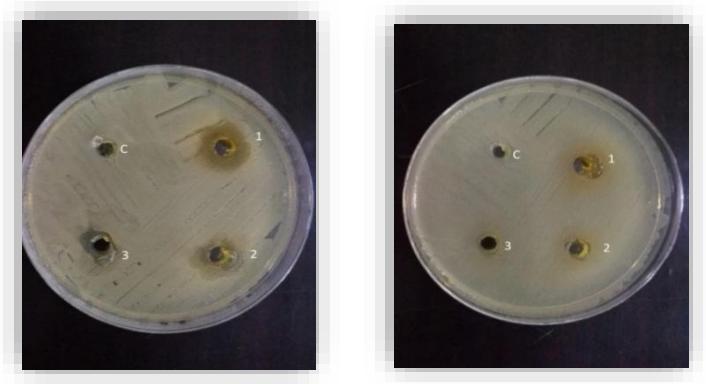
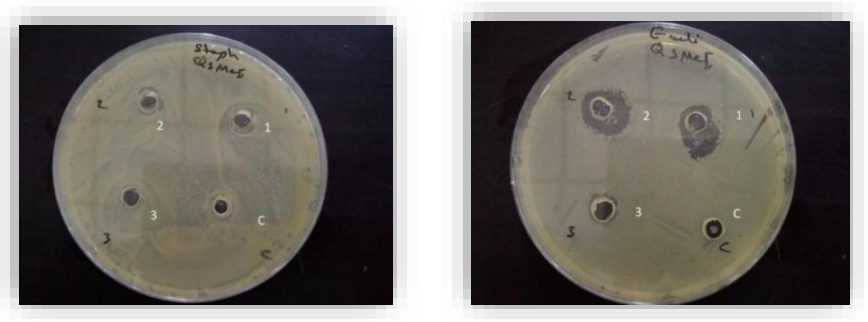


Figure 6. Quercetin activity against *Staphylococcus aureus* and where 1, 2, 3 and C represent 1, 0.5, 0.25 mg/mL and control solvent respectively.



(A) (B)

Figure 7. Compound 3 activity against (A) *Staphylococcus aureus* and (B) *Escherchia coli* where 1, 2, 3 and C represent 1, 0.5, 0.25 mg/ml and control solvent.



(A) (B)

Figure 8. Compound 4 activity against (A) *Staphylococcus aureus* and (B) *Escherchia coli* where 1, 2, 3 and C represent 1, 0.5, 0.25 mg/ml and control solvent.

4. Conclusions

On the basis of our results we conclude that at tested concentrations quercetin show superior antioxidant and antibacterial activity compared to its 1-6 derivatives, but, however, compound 3 and 4 *in vitro* antibacterial activity results reveal significant improvement in the gram-negative antibacterial activity against *Escherichia coli* as compared to that of quercetin.

Funding

This research received no external funding.

Acknowledgments

This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

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