DOI:10.5138/09750185.2302

AR JOURNALS ADVANCED RESEARCH

International Journal of Phytomedicine
2019;11(2):032-039

RESEARCH ARTICLE

Synthesis and Characterization of Novel Rutin Derivatives with Potential Antioxidant Properties

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Abstract

Rutin, a flavonol glycoside isolated from the fruit peels of Malus domestica family Rosaceae, is well known to possess antioxidant activity. This research was conducted in order to synthesize and characterize rutin derivatives and evaluate their antioxidant activities. Four derivatives were synthesized namely, Rutin-oxy-5, 7, 4' acetic acid **(2)**, Rutin -oxy- 5,7, 4' methyl benzoate amide **(3)**, 2", 2"', 2""-rutinoxymethyl-3-amino-1 -benzo[5,6-a] pyrimidine-4-one **(4)** and 5", 5"', 5""-tri-p-methoxybenzylideneamino-6-rutinoxy-benzo[5,6-a]-pyrimidin-4(5H)-one **(5) .** Their structures were elucidated using different spectral data (Mass, IR and ^{1}H, ^{13}C NMR). The antioxidant activities of rutin and its derivatives were evaluated by reducing power, Fe^{+2} chelating, DPPH* and ferric thiocyanate FTC assays. In addition, the results were compared with natural and synthetic antioxidants, such as α-tocopherol, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and trolox. Rutin and its derivatives were exhibited a strong reducing power, chelating activity on Fe^{2+} and free radical-scavenging. Antioxidant activity of rutin and its derivatives increased with increased concentrations. Total antioxidant activity of rutin, its derivatives and both standards decreased in the order of rutin > compound (5) > trolox > compound (2) > BHA > compound (4) > compound (3). This study showed that Rutin and its derivatives exhibited antioxidant activity in all tests and could be considered as a source of natural and synthetic antioxidants.

Keywords: Malus domestica; Rutin; Reducing power assay Fe +2 chelating assay DPPH assay Ferric thiocyanate assay

Introduction

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside: Figure 1) is a flavonoid of the flavonol-type that is widespread in the plant kingdom [1]. Rutin has a wide range of pharmacological properties (e.g., antioxidative activity) that have been exploited in human medicine and nutrition. Conventionally, it is used as an antimicrobial, antifungal, and anti-allergic agent. However, current research has shown its multi-spectrum pharmacological benefits for the treatment of various chronic diseases, such as cancer, diabetes, hypertension, and hypercholesterolemia [2]. Rutin exerts anti-inflammatory effects in ultraviolet β-irradiated mouse skin by inhibiting the expression of cyclooxygenase-2 and inducible nitric oxide synthase [3].

On our going medicinal chemistry research program we found that pyrimidine derivatives exhibit potent anticancer [4–12] and antibacterial [13–15] activities. Also, pyrimidine derivatives were found to possess several pharmacological properties, including antiviral [16], antifungal [17], hypoglycemic [18] and diuretic [19]activities. As an extension of our studies on the synthesis of some new biologically active heterocyclic compounds [18, 19], we now wish to report the synthesis of combining form of these two structural features into one molecule might produce compounds with promising antioxidant effects.

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Received: 01 Oct 2018, Accepted:18 Dec 2018

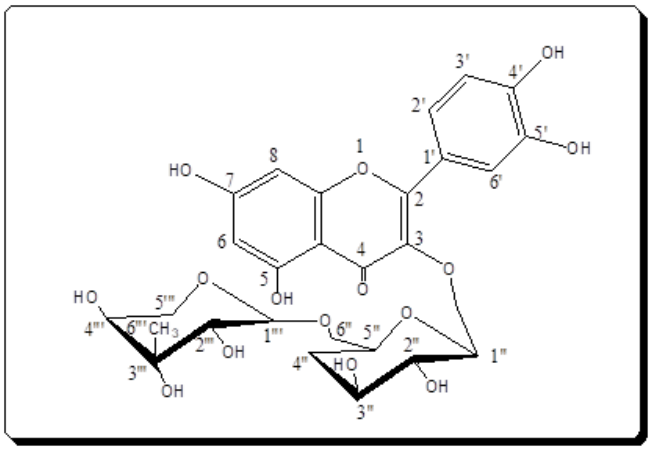

Figure 1 Chemical structure of rutin

Experimental

Plant Material

Apple fruits (Malus domestica Borkh. nom. illeg., cultivar Anna, family Rosaceae) were collected from the Faculty of Agriculture eco-station, Faculty of Agriculture Cairo University. and authenticated by the Vegetables and fruits department, Faculty of Agriculture, Cairo University. Their peels were cleaned, scraped off, then dried in shade, in a clean dust free environment at room temperature for several days and then grinded as powder. The powder obtained was stored in an air-tight container.

Chemicals and Reagents

All chemicals were purchased from Sigma Chemicals Co., St. Lious, MO, USA. Precoated TLC plates silica gel 60 GF_{254} (20 x 20 cm) (E-Merck), polyamide for column chromatography 6, (Fluka) and sephadex LH-20 for column chromatography (Pharmazia)

Apparatus

Rotatory evaporator, Heidolph, (G. Switzerland) was used for distilling off the different solvents. Melting points were determined in open capillaries and are uncorrected. ^{1}H- and ^{13}CNMR spectra were recorded in DMSO-d6 and CD_3OD, respectively, on a JEOL ECA-500 (^{1}H, 500 mHz and ^{13}C, 125 mHz), National Research Center, Giza, Egypt. UV-visible spectrophotometer, Shimadzu UV (P/N 204-58000) was used for all spectrophotometric assays. Melting points were determined on Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu MR 470 infrared spectrophotometer using the KBr pellets.

Extraction and isolation

Three hundred and twenty grams of the powdered apple peels were macerated in 70% aqueous ethanol till exhaustion. The hydro-ethanolic extracts were concentrated under reduced pressure at a temperature not exceeding 50°C to yield 47 g dark brown dried extract. The dried residue was suspended in water and partitioned successively between water and petroleum ether (5×50ml), dichloromethane (5×50ml), ethyl acetate (5×50ml) and n-butanol (5×50ml). The solvents were distilled off to yield respective fractions of 8.3 g, 6.9 g, 12.4 g and 5.2 g. The ethyl acetate fraction was further fractionated over a column chromatography packed with 200 gm polyamide as a stationary phase. The elution process started with distilled water, followed by gradient decrease in the polarity by adding methanol, starting with 10% up to 100% methanol. Fractions were collected each 25 ml and monitored by TLC on silica gel pre-coated plates using (ethyl acetate-methanol-water 100:16.5:13.5 v/v/v) as a mobile phase. The spots were visualized under UV light before and after exposure to ammonia vapors and spraying with aluminum chloride reagent. Similar fractions were pooled and separately concentrated under reduced pressure at a temperature not exceeding 50^{o}C. The water-methanol 7:3 eluted sub-fraction was subjected to sephadex column chromatography and further eluted with a stepwise gradient elution of water and methanol which resulted in the isolation of Compound 1 (31 mg). Its structure was elucidated by spectroscopic means.

Synthesis of rutin derivatives

Synthesis of Rutin-oxy-5, 7, 4' acetic acid (2)

A mixture of rutin (0.01 mol) and 0.03M NaOH in ethanol/water (2:8 v/v) was refluxed for 4hr. After cooling 0.03 M chloroacetic

acid was add drop-wise, the solid obtained was recrystallized from ethanol to give **(2).**

Synthesis of Rutin -oxy- 5,7, 4' methyl benzoate amide (3)

To a solution of Rutin-oxy-5, 7, 4' acetic acid **(2)** (0.01 mol) and methyl anthranilate (6.93 g, 0.03 mol) in xylene (50 ml), phosphorus trichloride (3 ml) was added. The reaction mixture was heated under reflux for 3–4 h. The crude product was recrystallized from ethanol to give **(3).**

Synthesis of 2", 2"', 2""-rutinoxymethyl-3-amino-1 -benzo[5,6-a]pyrimidine-4-one (4)

A mixture of Rutin -oxy- 5, 7, 4' methyl benzoate amide **(3)** (0.01 mol) and hydrazine hydrate (95 %) (0.15 mol) were dissolved in n-butanol (30 ml) and refluxed for 3–5 h. The solvent was concentrated and the residue was recrystallized from ethanol to give **(4)**.

Synthesis of 5", 5"', 5""-tri-p-methoxybenzylideneamino-6-rutinoxy-benzo [5, 6-a]-pyrimidin-4(5H)-one (5)

A mixture of 2", 2"', 2""-rutinoxymethyl-3-amino-1 -benzo[5,6-a]pyrimidine-4-one **(4)** (0.01 mol) and p-anisaldehyde (0.03 mol) in n-butanol was refluxed for 4hr. The reaction mixture was poured onto ice water and the solid obtained was recrystallized from dioxane to give **(5).**

Biological Investigations

Determination of antioxidant activity by reducing power assay

The reducing power of compounds 1-5 were measured according to the method of Oyaizu [20]. Various concentrations of compounds 1-5 (40–120 μg) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 7.6) and 2.5-ml potassium ferricyanide [K_3 $Fe(CN)_6$] (1 %, w/v), and then the mixture was incubated at 50^{o}C for 30 min. Afterward, 2.5 ml of trichloroacetic acid (10%, w/v) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. Finally, 2.5 ml of upper-layer solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1 %, w/v), and the absorbance was measured at 700 nm. α- tocopherol, BHA and BHT were used as standard antioxidants. Higher absorbance of the reaction mixture indicated greater reducing power.

Determination of antioxidant activity by Fe^{+2} chelating assay

The chelating activity of compounds (1-5) on ferrous ions (Fe^{+2}) were measured according to the method of Decker and Welch [21]. Aliquots of 1 ml of different concentrations (0.50, 1.0 and 1.5 mg/ml) of the samples were mixed with 3.7 ml of deionized water. The mixture was incubated with $FeCl_2$ (2 mM, 0.1 ml) for 10, 30, and 60 min. After incubation, the reaction was initiated by addition of ferrozine (5 mM and 0.2 ml) for 10 min at room temperature, and then the absorbance was measured at 562 nm in a spectrophotometer. A lower absorbance indicates a higher chelating power. The chelating activity of compounds (1-5) on Fe^{+2} were compared with that of EDTA at a level of 0.037 mg/ml. Chelating activity was calculated using the following formula:

Chelating activity (%) = [1- (Abs of sample/Abs of control) x 100]

Control test was performed without addition of compounds 1-5.

Determination of antioxidant activity by DPPH assay

The free radical-scavenging activity of compounds 1-5 were measured with 1, 1-diphenyl-2-picrylhydrazil ($DPPH^{\bullet}$) using the slightly modified methods of Brand-Williams et al. [22] and Takashira and Ohtake [23]. Briefly, 6 x10^{-5} mol/l $DPPH^{\bullet}$ solution in ethanol was prepared and 3.9 ml of this solution was added to 0.1 ml of the compounds 1-5 (2, 4 and 6 mg/ml) and trolox solution (0.02, 0.04 and 0.06 mg/ml). The mixture was shaken vigorously and the decrease in absorbance at 517 nm was measured at 10, 30, and 60 min. Water (0.1 ml) in place of compounds 2 and 3 were used as control. The percent inhibition activity was calculated using the following equation:

Inhibition activity (%) = [(A_o- A_1)/ A_o x 100].

Where A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of compounds 1-5 samples

Determination of antioxidant activity by ferric thiocyanate FTC assay

The antioxidant activity was determined according to the thiocyanate method of Osawa and Namiki [24] with slight modifications. For the stock solution, 10 mg of compounds 1-5 was dissolved in 10 ml water. Then, the solution of compounds 1-5 or standards samples (trolox and BHT) [100 mg/l] in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.6) was added to 2.5 ml of linoleic acid emulsion. Fifty-ml linoleic acid emulsion contained Tween 20 (175 μ), linoleic acid (155 μl), and potassium phosphate buffer (0.04 M, pH 7.6). On the other hand, 5.0 ml of control contained 2.5 ml of linoleic acid emulsion and 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.6). Each solution was then incubated at 37^{o}C in a glass flask in the dark. At 24 h intervals during incubation, 0.1 ml of this incubation solution was added to 4.7 ml of 75 % (v/v) ethanol and 0.1 ml of

30 % (w/v) ammonium thiocyanate. Precisely, 3 min after 0.1 ml of 0.02 M $FeCl_2$ in 3.5 % (w/v) HCl was added to the reaction mixture, the absorbance of the red color was measured at 500 nm in a spectrophotometer. The solutions without added compounds 1-5 or standards were used as the control. The inhibition of lipid peroxidation in percentage was calculated by the following equation.

Inhibition activity (%) = $[(A_o - A_1)/ A_o \times 100]$.

Where A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of compounds 1-5 or standards.

Statistical analysis

All the grouped data were statistically evaluated with SPSS/15 software [25]. Hypothesis testing methods included one-way analysis of variance (ANOVA). $P < 0.05$ was considered to indicate statistical significance. All the results were expressed as mean $\pm$ SD for six replicates.

Results and discussion

Structure elucidation of the isolated rutin (1)

Yellowish powder obtained from ethyl acetate fraction. The structure of isolated rutin with melting point of 230-232°C is presented in Figure 1. IR spectrum of rutin showed that 3365, 3210 (O-H stretching), 1510(C=O groups) and 1390(C-OH vibrations); ^{1}HNMR spectrum of rutin displayed the characteristic 3.02-3.6 (m,12H of sugar moieties), 4.3 (d, J=1.15Hz, 1H-Rham), 0.95 (3H, d,J=6Hz, CH_3-Rham), 6.1 (4H,d, J=7.8Hz, H-1 Glu), 5.3 (1H,d,=2Hz H-6), 6.2 (1H, d, J=2Hz, H-8), 6.7 (1H, d, J =8Hz, H- 5'), 6.99, 7.4, 6.93 (1H, , H-2', H-6'). The ^{13}CNMR spectrum showed the presence of specific signals (aromatic carbon, aliphatic chain and methoxy group) in agreement with the proposed structure of rutin. Also, the ^{13}CNMR spectrum showed the presence of 161.73 (C-2), 133.8 (C-3), 177.9(C-4), 164.6 (C-5), 104.49(C-6), 168.5(C-7), 94.12 (C-8), 156.9(C-9), 101.7(C-10), 121.7(C-1'), 116.3(C-2'), 145.6(C-3'), 148.6(C-4'), 122.1(C-5'), 126.2(C-6'), 99.2(C-1"), 68.8(C-2"), 76.4(C-3"), 74.6(C-4"), 72.3(C-5"), 71.06(C-6"), 40.1(C-1"'), 40.00(C-2"'), 39.8(C-3"'), 35.18(C-4"'), 32.8(C-5"'), 24.7 (C-6"").

Structure elucidation of Rutin derivatives compounds (2-5)

Compound 2

Preparation of the target compound Rutin-oxy-5, 7, 4' acetic acid (**2**) were achieved by the route depicted in Scheme 1. The structure of compound (**2**) was proved on the basis of elemental analyses, IR, ^{1}H NMR and ^{13}C NMR spectral data. The IR spectrum of compound (2) showed bands at 3400 (O-H stretching), 2916, 2819 (CH_2-stretching), 2664 (C-H bonding) and 1701, 1678, 1585, 1541(4C=O groups). ^{1}H NMR spectrum of compound (**2**) in ($DMSOd_6$) exhibited signals at 3.2-3.5(m,12H of sugar moieties), 3.70(d, J=1.15Hz, 1H-Rham), 1.52(3H,d,J=6Hz, CH_3-Rham), 6.3(4H,d, J=7.8Hz, H-1 Glu), 6.31 (1H,d,=2Hz H-6), 7.1 (1H, d, J=2Hz, H-8), 7.4 (1H, d, J =8Hz, H- 5'), 7.45, 7.46, 7.5 (1H, , H-2', H-6'), 10.3 (s,1H,carboxylic proton). ^{13}C NMR spectrum of compound (**2**) in (DMSOd6) exhibited signals at 156.20 (C-2), 124.84(C-1'), 114.6(C-2'), 140.42(C-3'), 145.5(C-4'), 127.82(C-5'), 129.79(C-6'), 101.0(C-1"), 48.6(C-2"), 40.7(C-3"), 40.5(C-4"), 40.71(C-5"), 40.34(C-6"), 40.2(C-1"'), 39.9(C-2"'), 39.5(C-3"'), 35.18(C-4"'), 32.8(C-5"'), 24.7 (C-6"").

Compound 3

The IR spectrum of compound **3** showed bands at 3390 (O-H stretching) and 1725, 1701, 1690, 1662, 1624, 1600, 1535(7C=O groups). ^{1}H NMR spectrum of compound **3** in ($DMSOd_6$) exhibited signals at 3.12-3.6, 3.80, 1.04 and 2.46 indicated that the presence of sugar moieties, 1H-Rham, CH_3-Rham, H-1 Glu. and $COCH_3$, respectively, as well as the absence of carboxylic protons. ^{13}C NMR spectrum of compound **3** in ($DMSOd_6$) exhibited signals at 182.47 (C-2), 123.37(C-1'), 119.47(C-2'), 147.26(C-3'), 140.5(C-4'), 125.4(C-5'), 130.0(C-6'), 95.30(C-1"), 70.07, 68.86, 56.9(3 methoxy groups), 40.12(C-2"), 39.95(C-3"), 39.7(C-4"), 38.7(C-5"), 35.47(C-6"), 33.65(C-1"'), 34.60(C-2"'), 30.54(C-3"'), 29.8(C-4"'), 32.8(C-5"'), 18.35 (C-6"").

Compound 4

The IR spectrum of compound **4** showed bands at 3398 (O-H stretching), 3178, 3274, 3124 cm^{-1} (NH_2) and 1732, 1658, 1690, 1620 (4C=O groups). ^{1}H NMR spectrum of compound **4** in ($DMSOd_6$) exhibited signals at 3.3-3.7, 3.97, 1.13, 6.73 and 6.73, 7.00 indicated that the presence of sugar moieties, 1H-Rham, CH_3-Rham, H-1 Glu. and NH_2, respectively, as well as the absence of $COCH_3$ group. ^{13}C NMR spectrum of compound **4** in ($DMSOd_6$) exhibited signals at 200.9 (C-2), 112.6(C-1'), 108.24(C-2'), 135.7(C-3'), 134.9(C-4'), 125.6(C-5'), 116.47(C-6'), 61.3(C-1"), 51.8(C-2"), 56.3(C-3"), 39.69(C-4"), 29.7(C-5"), 23.00(C-6"), 21.3(C-1"'), 24.52(C-2"'), 20.44(C-3"'), 19.7(C-4"'), 17.7(C-5"'), 14.01 (C-6"").

Compound 5

The IR spectrum of compound **5** showed bands at 3390 (O-H stretching), 2923, 2870 (CH_2-stretching), 2568 (C-H bonding), 1700, 1635, 1596, 11562(4C=O groups) and 1350 (C-OH vibrations) and indicated that the absence of NH_2 group. ^{1}H NMR

spectrum of compound **5** in ($DMSOd_6$) exhibited signals at 3.4-3.7, 4.1, 1.2 and 6.98 indicated that the presence of sugar moieties, 1H-Rham, CH_3-Rham. and H-1 Glu. as well as the absence of NH_2 signal, respectively. Also, ^{1}H NMR spectrum indicated that the absence of signal at 6.2 [s, 1H, N=CH, proton]. ^{13}C NMR spectrum of compound **5** in ($DMSOd_6$) exhibited signals at 176.03 (C-2), 120.9(C-1'), 96.6(C-2'), 143.7(C-3'), 130.5(C-4'), 116.9(C-5'), 115.8(C-6'), 71.9(C-1"), 56.17(C-2"), 53.9(C-3"), 40.15(C-4"), 39.98(C-5"), 39.82(C-6"), 27.18(C-1"'), 24.52(C-2"'), 23.5 (C-5") , 22.65 (C-5"') and 20.7(3C-5"') indicated that the presence of 3 double bond at [N=CH], 18.98 (C-6"").

Antioxidant activities

It is well known from the literature that the tested rutin ant it's derivatives exhibit a wide range of biological activities. So, it was of interest to design new compounds containing both these biologically active phenolic moieties and to study their antioxidant activities.

Figure 2 shows the reducing power of compounds 1-5. The reducing power of compounds 1-5 increased with increasing concentration. Based on a comparison of the absorbance at 700 nm, the reducing power of compounds 1 and 5 at a concentration of 40 μg/ml were nearly similar to that of BHT. This indicates that compounds 1 and 5 were electron donors and could also react with free radicals, converting them to more stable products and terminate the radical chain reaction. Also, 120 μg/ml compounds 1-5 are the best concentration which exhibits the most reducing power. In the reducing power assay, the presence of reductants (antioxidants) resulted in the reduction of the Fe^{+3}/ferricyanide complex to the ferrous form (Fe^{+2}) table 1. The amount of Fe^{+2}complex can therefore be monitored by measuring the formation of Perl's Prussian Blue at 700 nm. The reducing power of compounds 1-5 and both standards decreased in the order of compound 1> compound 5> BHT > compound 2 > compound 4 > α-tocopherol > BHA> compound 3. Table 2 shows the chelating effect of compounds 1-5. All samples at 1.50 mg/ml concentration showed the best chelating effect 48.87, 33.5, 20.8, 34.3 and 43.89% on ferrous ions at an incubation time of 60 min, respectively. The chelating activity of samples increased with increasing incubation times with $FeCl_2$. However, the chelating activity of compounds 2 of 1.25 mg/ml (30 %) was lower than EDTA at 0.037 mg/ml (43.67 %) for an incubation time of 90 min. This indicates that the chelation property of the compounds 1-5 on Fe^{+2} ions may afford protection against oxidative damage. The results in table 2, indicated that a significant property of compounds 1-5 is its capability for blocking the oxidative activity of systems with transition metal ion (Fe^{+2}/Fe^{+3}) that play an essential role in the formation of reactive oxygen species in Fenton's reactions.

The DPPH* radical-scavenging effects of compounds 1-5 are presented in Figure. 2 and showed appreciable free radical-scavenging activities. The free radical-scavenging activity of compounds 1-5 were compared to trolox, as a synthetic antioxidant. Compounds 1-5 of 6 mg/ml had the highest radical-scavenging activity when compared with 0.06 mg/ml trolox. The effects of 100 mg/l of compounds 1-5 on peroxidation of linoleic acid emulsion are shown in Figure. 2.

Compound 1(0.06mg/ml) showed higher antioxidant activity when compared to trolox, BHA, and BHT. Total antioxidant activity of compounds 1-5 and both standards decreased in the order of compound 1> compound 5> trolox > compound 4 > compound 2 > compound 3. Free radicals are known to be a major factor in biological damages, and DPPH$^\bullet$ has been used to evaluate the free radical-scavenging activity of natural antioxidants [26]. DPPH$^\bullet$, which is a molecule containing a stable free radical with a purple color, changes into a stable compound with a yellow color by reacting with an antioxidant which can donate an electron to DPPH$^\bullet$. In such case, the purple color typical of the free DPPH$^\bullet$ radical decays, a change which can be followed either spectrophotometrically (517 nm). The proton radical-scavenging action is known as an important mechanism of antioxidation. 1, 1-Diphenyl-2-picrylhydrazil (DPPH$^\bullet$) is used as a free radical to evaluate the antioxidative activity of some natural sources [27]. The DPPH$^\bullet$ radical-scavenging effects of compounds 1-5 are presented in Fig. 3. From these results, it can be stated that compounds 1 and 5 have the ability to scavenge free radicals and could serve as a strong free radical inhibitor or scavenger according to trolox. Many attempts at explaining the structure–activity relationships of some phenolic compounds have been reported in the literature. It has been reported that the antioxidant activity of phenolic compounds may result from the neutralization of free radicals initiating oxidation processes, or from the termination of radical chain reactions, due to their hydrogen donating ability [28]. It is also known that the antioxidant activity of phenolic compounds is closely associated with their structures, such as substitutions on the aromatic ring and side chain structure. Their accessibility to the radical center of DPPH$^\bullet$ could also influence the order of the antioxidant power. Free radical scavenging activity of phenolic compounds is believed to be influenced by the number and position of phenolic hydrogen in their molecules [29]. It is also proposed that the higher antioxidant activity of compounds 1-5 is related to the presence of hydroxyl groups [30].

Table 1 Reducing power of compounds 1-5, BHA, BHT and α-tocopherol

		Concentrations				
	Compounds	40 ug/ml	60 ug/ml	80 ug/ml	100 ug/ml	120 ug/ml
Absorbance	1	0.43 ± 0.02^{Aa}	0.45 ± 0.02^{Aa}	0.54 ± 0.01^{A}	0.62 ± 0.02^{A}	0.76 ± 0.02^{A}
	2	0.28 ± 0.01^{Ba}	0.32 ± 0.02^{B}	0.42 ± 0.04^{Ba}	0.50 ± 0.03^{B}	0.51 ± 0.03^{B}
	3	0.19 ± 0.03^{Ca}	0.21 ± 0.01^{Ca}	0.32 ± 0.02^{Cb}	0.36 ± 0.02^{Cb}	0.40 ± 0.02^{C}
	4	0.26 ± 0.02^{B}	0.35 ± 0.02^{Ba}	0.37 ± 0.01^{Ca}	0.44 ± 0.04^{Bb}	0.48 ± 0.04^{Bb}
	5	0.46 ± 0.01^{A}	0.53 ± 0.03^{Aa}	0.58 ± 0.03^{Aa}	0.64 ± 0.03^{Ab}	0.69 ± 0.02^{Ab}
	α- tocopherol	0.33 ± 0.05^{Ba}	0.34 ± 0.01^{Ba}	0.39 ± 0.02^{C}	0.46 ± 0.02^{Bb}	0.45 ± 0.01^{Cb}
	BHT	0.45 ± 0.03^{Aa}	0.48 ± 0.02^{Aa}	0.46 ± 0.04^{Ba}	0.50 ± 0.01^{Bb}	0.53 ± 0.04^{Bb}
	BHA	0.25 ± 0.04^{Ba}	0.27 ± 0.03^{Ca}	0.34 ± 0.02^{Cb}	0.39 ± 0.01^{Cb}	0.41 ± 0.02^{C}

Values are given as mean $\pm$ SD for six replicates
Data followed by the same letter are not significantly different at$P \leq 0.05$. Small letters are used for comparison between the means within thecolumns. Capital letters are used to compare means within the rows.

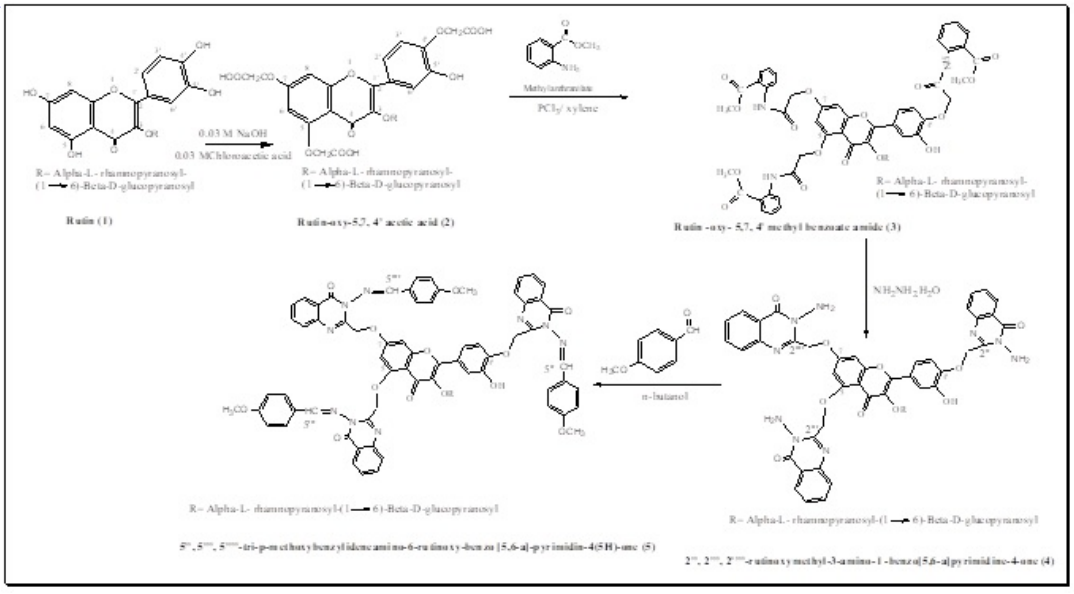

Figure 2 Rationalized formation of compounds 1-5

Table 2 Chelating effects of different concentrations of compounds 1-5 on Fe^{+2} ions at different incubation times with $FeCl_2$.

		Time (min)				
	Compounds	10 min.	30 min.	60 min.	90 min.	120 min.
Chelating activity (%)	1	15.50 ± 1.5^{A}	30.98 ± 1.5^{A}	48.87 ± 3.18^{A}	53.30 ± 2.97^{Aa}	53.3 ± 4.3^{Aa}
	2	10.38 ± 0.98^{B}	25.48 ± 2.45^{B}	33.50 ± 2.04^{Ba}	36.09 ± 2.77^{a}	36.99 ± 3.25^{a}
	3	6.72 ± 0.67	12.76 ± 2.14	20.85 ± 3.27^{a}	22.11 ± 3.00^{a}	23.65 ± 2.61^{a}
	4	9.86 ± 0.9^{B}	21.50 ± 2.71^{B}	34.30 ± 3.08^{B}	44.28 ± 4.22^{a}	46.90 ± 2.59^{a}
	5	18.11 ± 2.06^{A}	29.96 ± 2.76^{A}	43.89 ± 4.07^{A}	49.11 ± 3.80^{Aa}	50.25 ± 3.97^{Aa}

Values are given as mean $\pm$ SD for six replicates
Data followed by the same letter are not significantly different at$P \leq 0.05$. Small letters are used for comparison between the means within thecolumns. Capital letters are used to compare means within the rows.

Conclusion

In conclusion, rutin when combining with quinazoline derivatives exhibited promising antioxidant activity. More studies are needed to prove their medicinal and biological importance which may pave the way for possible therapeutic applications.

Conflicts of interest

The authors declare no conflicts of interest.

Author Disclosure Statement

No competing financial interests exist.

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