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Isolation and Antimicrobial Activity of Rutin and its Derivatives from *Ruta chalepensis* (Rutaceae) Growing in Iraq

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Abstract: Rutin (1), rutin 3'-methyl ether (2), and a new flavonol glycoside, 6-hydroxy-rutin 3',7-dimethyl ether (3), were isolated from the methanol extract of the fruits of *Ruta chalepensis*, collected from Diyala, Iraq. Their structures were elucidated by spectroscopic analyses, including 1D-, 2D-NMR and HRESIMS. Antimicrobial activity of compounds 1-3 was tested against four Gram +ve and Gram -ve bacterial strains, and the only fungal strain, *Candida albicans*, using the 96-well based resazurin microtitre assay.

Keywords: *Ruta chalepensis*; Rutaceae; resazurin assay; antimicrobial activity; rutin; 6-hydroxy-rutin 3',7-dimethyl ether. © 201X ACG Publications. All rights reserved.

1. Introduction

Ruta chalepensis L. (family: Rutaceae), commonly known as “fringed rue”, is a perennial herb (ca. 80 cm tall), native to Eurasia and North Africa and introduced elsewhere [1-4]. Different parts of this plant have been used for centuries in traditional medicine to treat convulsions, dropsy, fever, mental disorders, menstrual problems, neuralgia, rheumatism, and other bleeding and nervous disorders [2, 4-6]. Whilst previous phytochemical studies on this plant revealed the presence of several alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, tannins and terpenoids [3, 4, 7-10], pharmacological evaluations established its analgesic, anthelmintic, anti-acetylcholinesterase, anticancer, anti-inflammatory, antimicrobial, antioxidant and antiparasitic properties [1, 5-8, 11-15]. We now report on the isolation, characterization and antimicrobial activity of three flavonol glycosides (1-3) (Figure 1) including a new one, 6-hydroxy-rutin 3',7-dimethyl ether (3) from the fruits of *R. chalepensis*, collected in Iraq.

2. Materials and Methods

2.1. General

Chromatographic solvents were purchased from Fisher Scientific, UK, and used without further purification. The NMR spectroscopic analyses were performed on a Bruker AMX600 NMR spectrometer (600 MHz for ¹H, and 150 MHz for ¹³C). MS analyses were performed on a Xevo G2-S ASAP or LTQ Orbitrap XL 1 spectrometers. UV spectra were recorded on Analytik Jena Specord 210 spectrophotometer. The SPE fractions were analysed on a Dionex Ultimate 3000 UHPLC coupled with a photodiode array (PDA) detector, using a Phenomenex Gemini-NX 5 U C₁₈ column (150 × 4.6 mm, 5 μm, Phenomenex, USA) and gradient solvent systems comprising MeOH (solvent B) and water

(solvent A) (both contained 0.1% TFA, flow rate: 1 mL/min) were employed to optimize separation of compounds for preparative HPLC. An Agilent 1260 Infinity series preparative HPLC coupled with a PDA detector was used to isolate compounds on an ACE preparative column (150 × 21.2 mm, 5 μm, Hichrom Ltd, UK; MeOH-water linear gradient, flow rate: 10 mL/min). The column temperature was set at 25 °C. The chromatogram was monitored at variable UV–vis wavelengths (215, 254, 280 and 320 nm).

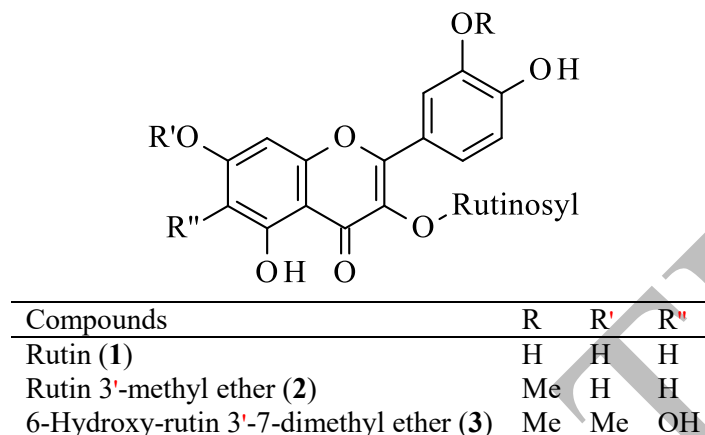


Figure 1. Structures of flavonol glycosides **1-3**

2.2 Plant Materials

The fruits of *R. chalepensis* L. were collected from Diyala, Central Iraq (N 33.79684 E 44.623337) in September 2015, air-dried at room temperature, and ground using a coffee grinder. A voucher specimen (33396) for this collection has been retained at the National Herbarium of Iraq.

2.3. Extraction and Isolation

Ground air-dried fruits (103 g) were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane (DCM) and methanol (MeOH) (0.9 L, 10 cycles each). All extracts were evaporated to dryness using a rotary evaporator at 45°C to obtain 4.6 g, 4.2 g and 6.0 g of *n*-hexane, DCM and MeOH extracts, respectively. A portion (2 g) of the active MeOH extract was subjected to solid-phase extraction (SPE) on a Strata (20 g, Phenomenex) C₁₈ reversed-phase cartridge eluted with water-MeOH mixture of decreasing polarity, water:MeOH 80:20, 50:50, 20:80 and 0:100 (250 mL each), to obtain four SPE fractions I-IV, respectively. All SPE fractions were dried using a rotary evaporator followed by freeze-drying and stored in sealed vials in a fridge for further work. Reversed-phase preparative HPLC purification of the SPE fraction II (linear gradient: solvent B in A, 30-100% in 30 min) afforded compounds **1** (3.5 mg, *t_R* = 15.9 min), **2** (2.1 mg, *t_R* = 17.1 min) and **3** (3.2 mg, *t_R* = 18.0 min), respectively.

2.3.1. Rutin (**1**)

Yellow amorphous solid; UV λ_{\max} (MeOH) nm: 257, 267 (sh), 300 (sh) and 359 nm; ¹H NMR (CD₃OD, 600 MHz), see Table 1; ¹³C NMR (CD₃OD, 150 MHz), see Table 2; HRESIMS (negative ion mode) *m/z* 609.1456 [M-H]⁻, (calcd. for C₂₇H₂₉O₁₆, *m/z* 609.1456) [16].

2.3.2. Rutin 3'-methyl ether (**2**)

Brown amorphous solid; UV λ_{\max} (MeOH) nm: 254, 264 (sh), 304 (sh) and 354 nm; ¹H NMR (CD₃OD, 600 MHz), see Table 1; ¹³C NMR (CD₃OD, 150 MHz), see Table 2; HRESIMS (positive ion mode) *m/z* 625.1761[M+H]⁺ (calcd. for C₂₈H₃₃O₁₆, *m/z* 625.1769) [16-18].

2.3.3. 6-Hydroxy-rutin 3',7-dimethyl ether (**3**)

Brown amorphous solid; UV λ_{\max} (MeOH) nm: 252, 260 (sh), 306 (sh) and 360 nm; ¹H NMR (CD₃OD, 600 MHz), see Table 1; ¹³C NMR (CD₃OD, 150 MHz), see Table 2; HRESIMS (positive ion mode) *m/z* 655.1870 [M+H]⁺ (calcd. for C₂₉H₃₅O₁₇, *m/z* 655.1874).

2.4. Antimicrobial Assay (Resazurin Assay)

All three compounds (**1-3**) were screened for their potential antimicrobial activity against four bacterial strains, e.g., Gram +ve: *Micrococcus luteus* (NCTC 7508) and *Staphylococcus aureus* (NCTC 12981) and Gram -ve: *Escherichia coli* (NCTC12241) and *Pseudomonas aeruginosa* (NCTC 12903), and a fungal strain, *Candida albicans* (ATCC 90028) using the resazurin 96-well microtitre plate-based *in vitro* antimicrobial assay [19]. All bacterial strains were cultured on nutrient agar (Oxoid) and incubated for 24h at 37°C to determinate the MIC. Ciprofloxacin was used as a positive control for bacterial strains, and nystatin for *C. albicans*. Resazurin solution, prepared by dissolving 1 mg of resazurin in 5 mL of sterile distilled water, was used in this assay as an indicator of cell growth, and the method was as described by Sarker *et al.* [19]. This experiment was performed under aseptic conditions using a sterile 96 well plate. Compound solution (100 µL) was added into the first row of the plate and 50 µL of normal saline was added to all other wells. Serial dilutions were achieved using a multichannel pipette. To each well, 30 µL of nutrient broth and 10 µL of resazurin indicator solution were added. Finally, 10 µL of bacterial suspension (5×10^5 cfu/mL) was added to each well. To prevent bacterial dehydration, each plate was wrapped loosely with cling film. Every plate contained a positive control. Any colour changes (or no changes in positive control), blue to pink, were noted. The lowest concentration was taken in the colour change that occurred as an MIC (minimum inhibitory concentration) value. The mean of three values was calculated.

2.4.1. Determination of bacteriostatic or bactericidal property

To determine whether the compounds were bacteriostatic or bactericidal, under aseptic condition, 100 µL of culture from the rows of MIC in the microtitre plates was transferred to an empty petri dish, 20 mL of nutrient agar was poured on to the dish, and incubated at 37°C for 24 h. Any microbial regrowth indicated bacteriostatic, and no growth indicated bactericidal activity of the test sample.

3. Results and Discussion

3.1. Extraction, Isolation and Structure Elucidation of Rutin Derivatives (**1-3**)

Soxhlet extraction of the dried and ground fruits of *Ruta chalepensis* afforded 4.6, 4.2 and 6.0 g of *n*-hexane, DCM and MeOH extracts, respectively. SPE of the MeOH extract, the most active extract of the three, produced 0.45, 0.49, 0.50 and 0.40 g of SPE fractions, eluted with 20, 50, 80 and 100% MeOH in water, indicating an excellent recovery of the sample from the SPE cartridge. Antimicrobial assay-guided and preparative HPLC-assisted purification of the most active SPE fraction II afforded compounds **1-3**. The UV-Vis spectral analyses of the compounds **1-3** indicated that they were flavonoid glycosides, possibly with rutin-type skeleton [18]. The HRESIMS analyses of these compounds (**1-3**) provided their molecular formula, $C_{27}H_{30}O_{16}$, $C_{28}H_{32}O_{16}$ and $C_{29}H_{34}O_{17}$, respectively, suggesting compound **1** could be rutin. It also added further evidence to the assumption made from the UV-Vis data that compounds **1-3** could be rutin derivatives. In fact, according to the HRESIMS data, compound **2** had just 14 amu unit more than that of rutin (**1**) suggesting this compound could be a methyl ether of rutin (**1**). Similarly, the molecular mass of compound **3** was 44 amu unit higher than that of rutin (**1**), indicating this compound could be a dimethyl ether analogue of **1** with an additional hydroxyl group. The 1H and ^{13}C NMR data (Tables 1 and 2) of compounds **1** and **2** were in good agreement with respective published data for rutin (**1**) and rutin 3'-methyl ether (**2**) [16-18]. The attachment of the rutosyl moiety at C-3 in compounds **1** and **2**, and the methyl ether formation at C-3' in compound **2** were confirmed from extensive HSQC and HMBC experiments.

The 1H NMR spectrum (Table 1) of compound **3** was quite similar to that of compound **2**, with the exception that there was a downfield 6H singlet at $\delta 3.97$, and instead of the usual shielded aromatic *meta*-coupled proton signals for H-6 and H-8 in flavonol skeleton, there was only a 1H singlet at $\delta 6.53$ suggesting that one of those protons was substituted by an extra functional group, most likely a hydroxyl group based on the HRESIMS data. The ^{13}C NMR spectrum of **3** (Table 2) showed the presence of an additional oxygenated aromatic quaternary signal at $\delta 145.3$, which could be assigned to C-6 or C-8. Whilst a COSY experiment of compound **3** established all major 1H - 1H scalar couplings, 1H - ^{13}C direct (1J) couplings were obtained from an HSQC experiment confirming the

assignment of all protonated ^{13}C signals to their respective ^1H signals. An HMBC experiment displayed all major ^1H - ^{13}C long-range (2J and 3J) correlations (Figure 2). The rhamnose anomeric proton (H-1''') showed a 3J correlation to C-6''' of the glucose unit, and the glucose anomeric proton (H-1'') displayed a 3J correlation to C-3 of the flavonol aglycones, confirming the formation of the disaccharide, rutinose, and its connection to C-3 (as in compounds **1** and **2**). Similarly, 3J correlations from the methoxyl signal at $\delta 3.97$ (6H) (Table 1) to C-7 and C-3' (Table 2) established methyl ether formation at C-7 and C-3' (Figure 2). The ^{13}C NMR (Table 2) signal at $\delta 96.4$ confirmed that the hydroxylation was indeed on C-6 in compound **3**, and this fact was further corroborated from the ^1H - ^{13}C HMBC long-range correlations from H-8 signal to C-6 and C-10 (Figure 2). Thus, the compound **3** was identified as 6-hydroxy-rutin 3',7-dimethyl ether (**3**), which to the best of our knowledge, is a new natural product. This is also the first report on any assay-guided phytochemical analysis of the fruits of Iraqi *R. chalepensis*.

Table 1. ^1H NMR data for compounds **1-3** (at 600 MHz in CD_3OD , δ in ppm, J in Hz)

Position (H)	1	2	3
<i>Aglycone</i>			
6	6.23 (1H, <i>d</i> , $J = 2.1$)	6.23 (1H, <i>d</i> , $J = 2.0$)	-
8	6.42 (1H, <i>d</i> , $J = 2.1$)	6.44 (1H, <i>d</i> , $J = 2.0$)	6.53 (1H, <i>s</i>)
2'	7.69 (1H, <i>d</i> , $J = 2.0$)	7.96 (1H, <i>d</i> , $J = 2.0$)	8.00 (1H, <i>d</i> , $J = 2.0$)
5'	6.89 (1H, <i>d</i> , $J = 8.4$)	6.94 (1H, <i>d</i> , $J = 8.4$)	6.94 (1H, <i>d</i> , $J = 8.4$)
6'	7.65 (1H, <i>dd</i> , $J = 2.0, 8.4$)	7.65 (1H, <i>dd</i> , $J = 2.0, 8.4$)	7.80 (1H, <i>dd</i> , $J = 2.0, 8.4$)
7-OMe	-	-	3.97 (3H, <i>s</i>)
3'-OMe	-	3.97 (3H, <i>s</i>)	3.97 (3H, <i>s</i>)
<i>Glucose moiety</i>			
1''	5.12 (1H, <i>d</i> , $J = 7.7$)	5.25 (1H, <i>d</i> , $J = 7.6$)	5.25 (1H, <i>d</i> , $J = 7.4$)
2''	3.49*	3.49*	3.49*
3''	3.31*	3.32*	3.31*
4''	3.29*	3.30*	3.29*
5''	3.43*	3.46*	3.43*
6''	3.41*	3.44*	3.44*
	3.81 (1H, <i>m</i>)	3.84 (1H, <i>m</i>)	3.84 (1H, <i>m</i>)
<i>Rhamnose moiety</i>			
1'''	4.54 (1H, <i>d</i> , $J = 1.5$)	4.55 (1H, <i>d</i> , $J = 1.5$)	4.54 (1H, <i>d</i> , $J = 1.4$)
2'''	3.65*	3.63*	3.60*
3'''	3.55*	3.56*	3.54*
4'''	3.30*	3.28*	3.28*
5'''	3.46*	3.43*	3.43*
6'''	1.14 (3H, <i>d</i> , $J = 6.2$)	1.11 (3H, <i>d</i> , $J = 6.2$)	1.11 (3H, <i>d</i> , $J = 6.3$)

*Overlapped peaks – confirmed from COSY. HSQC and HMBC experiments

3.2 Antimicrobial activity

Potential antimicrobial properties of rutin (**1**) and its two derivatives (**2** and **3**) were assessed against two Gram +ve, two Gram -ve bacterial species, and a fungal strain (Table 3). All compounds (**1-3**) showed various degrees of activities against all tested organisms in the resazurin microtitre assay [19]. Among three compounds, compound **3** was the most potent one, and the order of antimicrobial potency among these compounds was **3**>**2**>**1**, which indicated that the addition of methyl ether functionality on rutin (**1**) enhanced the potency; more the number of methoxyl groups, the better was the activity. In fact, the cell membrane is considered as a direct target of antibacterial action of flavonoids, suggesting that membrane interaction could be an important mechanism of antibacterial activity of flavonoids, and increased lipophilicity in flavonoids might lead to enhanced membrane interaction [20]. All three compounds were particularly active against the fungal strain *Candida albicans* (MICs = 96-205 μM). *Ruta chalepensis* has long been used in Iraqi traditional medicine to

treat various diseases including microbial infections [3,4]. Antimicrobial assay-guided isolation of rutin (**1**) and its derivatives (**2** and **3**) as active components and subsequent assessment of their antimicrobial properties have provided some scientific evidence for the traditional use of *R. chalepensis* to treat infections.

Table 2. ^{13}C NMR data for compounds **1-3** (at 150 MHz in CD_3OD , δ in ppm)

Position (C)	1	2	3
<i>Aglycone</i>			
2	159.5	159.0	159.3
3	135.7	135.6	135.3
4	179.5	179.5	180.0
5	163.2	163.2	153.2
6	100.0	100.1	127.8
7	166.2	166.2	155.5
8	95.0	95.0	96.4
9	158.5	158.7	145.3
10	105.7	105.9	106.0
1'	123.2	123.2	123.3
2'	117.8	114.7	114.8
3'	146.0	148.5	148.5
4'	150.4	151.0	151.0
5'	116.2	116.3	116.2
6'	123.7	124.2	124.7
7-OMe	-	-	57.2
3'-OMe	-	56.9	56.9
<i>Glucose moiety</i>			
1''	104.8	104.5	104.5
2''	75.9	76.1	76.1
3''	77.4	72.4	72.4
4''	71.5	77.5	77.5
5''	78.3	78.3	78.3
6''	68.7	68.1	68.7
<i>Rhamnose moiety</i>			
1'''	102.5	102.7	102.7
2'''	72.2	72.2	72.2
3'''	72.4	71.8	71.8
4'''	74.1	74.0	74.0
5'''	69.8	69.9	69.9
6'''	18.0	18.0	18.0

Table 3. Antimicrobial activity of compounds **1-3**

Test organisms	Minimum inhibitory concentration (MIC) in μM				
	Positive controls		Compounds		
	Ciprofloxacin	Nystacin	1	2	3
<i>Escherichia coli</i> (NCTC 12241)	1.473	N/A	1638.9	1601.2	774.5
<i>Micrococcus luteus</i> (NCTC 7508)	2.947	N/A	409.7	200.1	193.6
<i>Pseudomonas aeruginosa</i> (NCTC 12903)	7.36×10^{-1}	N/A	1638.9	800.6	387.3
<i>Staphylococcus aureus</i> (NCTC 12981)	2.947	N/A	1638.9	1601.2	1459.1
<i>Candida albicans</i> (ATCC 90028)	N/A	1.054	204.9	100.1	96.8

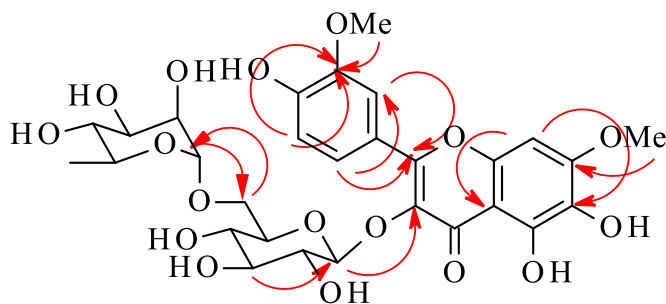


Figure 2. ^1H - ^{13}C HMBC selected long-range key correlations of compound **3**

Rutin (**1**) is one of the most common flavonol glycosides, especially found in the plants from the family Rutaceae, and has been extensively studied to establish its various bioactivities including antibacterial and antifungal properties [16, 21-23]. Therefore, the antimicrobial activities of rutin (**1**) and its analogues (**2** and **3**), including a new one (**3**), as observed in the present study, are in agreement with previous reports [16, 21-23]. All these compounds (**1-3**) were found to be bacteriostatic [24,25] as there was microbial regrowth, when under aseptic condition, 100 μL of culture from the rows of MIC in the microtitre plates was transferred to an empty petri dish, 20 mL of nutrient agar was added, and incubated at 37°C for 24 h.

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Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/RNP>

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References

- [1] I. Ouerghemmi, I. B. Rebey, F. Z. Rahali, S. Bourgou, L. Pistelli, R. Ksouri, B. Marzouk and M. S. Tounsi (2017). Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian *Ruta chalepensis*, *J. Food Drug Anal.* **25**, 350-359.
- [2] A. Pollio, A. De Natale, E. Appetiti, G. Aliotta, and A. Touwaide (2008). Continuity and change in the Mediterranean medical tradition: *Ruta* spp. (Rutaceae) in Hippocratic medicine and present practices, *J. Ethnopharmacol.* **116**, 469-482.
- [3] K. Günaydin and S. Savci, S (2003). Phytochemical studies on *Ruta chalepensis* (LAM.) Lamarck, *Nat. Prod. Res.* **19**, 203-210.
- [4] M. S. Al-Said, M. T. Yahia, S. R. Ginnawi and A. M. Ageel (1990). Studies on *Ruta chalepensis*, an ancient medicinal herb still used in traditional medicine, *J. Ethnopharmacol.* **23**, 305-312.
- [5] L. Aguilar-Santamaria, and J. Tortoriello (1995). Anticonvulsant and sedative effects of crude extracts of *Ternstroemia pringlei* and *Ruta chalepensis*, *Phytother. Res.* **10**, 531-533.
- [6] M. S. Ali-Shtayeh and S. I. Abu Ghdeib (1990). Antifungal activity of plant extracts against dermatophytes. *Mycoses.* **42**, 665-672.
- [7] M. E. Gonzalez-Trujano, D. Carrera, R. Ventura-Martinez, E. Cedillo-Portugal and A. Navarrete (2006). Neuropharmacological profile of an ethanol extract of *Ruta chalepensis* L. in mice, *J. Ethnopharmacol.* **106**, 129-135.

- [8] L. Iauk, K. Mangano, A. Rapisarda, S. Ragusa, L. Maiolino, R. Musumeci, R. Costanzo, A. Serra, A. and A. Speciale (2004). Protection against murine endotoxemia by treatment with *Ruta chalepensis* L. a plant with anti-inflammatory properties, *J. Ethnopharmacol.* **90**, 267-272.
- [9] K. El Sayed, M. S. Al-Said, F. S. El-Ferally and S. A. Ross (2000). New quinoline alkaloids from *Ruta chalepensis*, *J. Nat. Prod.* **63**, 995-997.
- [10] A. Emam, M. Eweis and M. Elbadry (2010). A new furoquinoline alkaloid with antifungal activity from the leaves of *Ruta chalepensis*, *Drug Dis. Therap.* **4**, 399-404.
- [11] M. Hamdiken, S. Bouhalit and Z. Kechrid (2017). Effect of *Ruta chalepensis* on zinc, lipid profile and antioxidant levels in the blood and tissue of streptozotocin-induced diabetes in rats fed zinc-deficient diets, *Can. J. Diabetes* DOI:10.1016/j.jcjd.2017.08.239.
- [12] E. Ortu, G. Sanna, A. Scala, G. Pulina, P. Caboni and G. Battacone (2017). *In vitro* anthelmintic activity of active compounds of the fringed rue *Ruta chalepensis* against dairy ewe gastrointestinal nematodes, *J. Helminthol.* **91**, 447-453.
- [13] A. Khadri, I. Bouali, S. Belkhir, R. Mokded, S. Smiti, P. Fale, M. E/ M. Araujo and M. L. M. Serralheiro (2017). *In vitro* digestion, antioxidant and antiacetylcholinesterase activities of two species of *Ruta*: *Ruta chalepensis* and *Ruta montana*, *Pharm. Biol.* **55**, 101-107.
- [14] M. Kacem, G. Simon, R. Leschiera, L. Misery, A. El-Feki and N. Lebonvallet (2015). Antioxidant and anti-inflammatory effects of *Ruta chalepensis* L. extracts on LPS-stimulated RAW 264.7 cells, *In Vitro Cell. Dev. Biol. Animal* **51**, 128-141.
- [15] R. M. Sghaier, D. Khelifi, M. Hamdi, J. Boujila and D. Laouini (2013). *Ruta chalepensis*: a promising phytotherapeutic candidate against multiple forms of cancer, *Eur. J. Cancer* **49**, S179.
- [16] F. Fathiazad, A. Delazar and S. D. Sarker (2006). Extraction of flavonoids and quantification of rutin from waste tobacco leaves, *Iranian J. Pharm. Res.* **3**, 222-227.
- [17] J. Kagan and T. J. Mabry (1969). Isorhamnetin 3-*O*-rutinoside, the flavonoid pigment in *Batis maritima*, *Phytochemistry* **8**, 325-326.
- [18] T. Mabry, K. R. Markham and M. B. Thomas (1970). *The Systematic Identification of Flavonoids*, Springer-Verlag, Berlin Heidelberg, pp. 1-354.
- [19] S. D. Sarker, Y. Kumarasamy and L. Nahar (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals, *Methods* **42**, 321-324.
- [20] T. Wu, M. He, X. Zang, Y. Zhou, T. Qiu, S. Pan and X. Xu (2013). Structure-activity relationship study of flavonoids as inhibitors of *E. coli* by membrane interaction effect. *Biochimica et Biophysica Acta*, **1828**, 2751-2756.
- [21] M. M. Almutairi, W. A. Alanazi, M. A. Alshammari, M. R. Alotaibi, A. R. Alhoshani, S. S. Al-Rejaie, M. M. Hafez and O. A. Al-Shabanah (2017). Neuro-protective effect of rutin against cisplatin-induced neurotoxic rat model. *BMC Complement. Alt. Med.* **17**, 472.
- [22] D. D. Orhan, B. Özçelik, B. Özgen and F. Ergun (2009). Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiol. Res.* **165**, 496-504.
- [23] R. B. D. Q. Pimentel, C. A. D. Costa, P. M. Albuquerque and S. D. Junior (2013). Antimicrobial activity and rutin identification of honey produced by the stingless bee *Melipona compressipes manaosensis* and commercial honey. *BMC Complement. Alt. Med.* **13**, 151.
- [24] S. Bernatová, O. Samek, Z. Pilát, M. Šerý, J. Ježek, P. Jákl, M. Šiler, V. Krzyžánek, P. Zemánek, V. Holá, M. Dvořáčková and F. Růžička, F. (2013). Following the mechanisms of bacteriostatic versus bactericidal action using raman spectroscopy. *Molecules* **18**, 13188-13199; doi:10.3390/molecules181113188
- [25] G. A. Pankey and L. D. Sabath (2004). Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clin. Infect. Dis.* **38**, 864-870.