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Studies on the chemical constituents and anticancer activity of *Saxifraga stolonifera* (L) Meeb

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Abstract—*Saxifraga stolonifera*, an evergreen dicotyledon, has been identified as an important resource in Chinese medicine due to its anticancer activity. In the present report, chemical components of *S. stolonifera* (L) Meeb which is found in Guizhou province were investigated. Ten compounds were isolated from ethanol extracts of *S. stolonifera* plant and were identified as *n*-$C_{31}H_{64}$ (**1**), (*n*-$C_{17}H_{35})_2CO$ (**2**), β-sitosterol (**3**), *n*-$C_{29}H_{60}$ (**4**), Bergenin (**5**), Protocatechuic acid (**6**), Gallic acid (**7**), Quercitrin 3-*O*-α-L-rhamnoside (**8**), Quercetin (**9**), and Quercetin 3-*O*-β-D-glucopyranoside (**10**). Among them, *n*-$C_{31}H_{64}$ (**1**), (*n*-$C_{17}H_{35})_2CO$ (**2**), β-sitosterol (**3**), and *n*-$C_{29}H_{60}$ (**4**) were isolated from this plant for the first time. The anticancer activities of *S. stolonifera* constituents on human gastric carcinoma cell line BGC-823 were evaluated by MTT assay and microscopic observation, DNA fragmentation, and flow cytometry analysis assay. It was found that quercetin could inhibit cell viability after 72 h of exposure. Furthermore, DNA ladder assay revealed that quercetin could induce DNA strand break in a concentration- and time-dependent fashion. Flow cytometric analysis shows that quercetin can induce 11.82% BGC-823 cell apoptosis in 48 h. These data reveal that quercetin is a potential agent capable of inducing apoptosis in BGC-823 cells.

1. Introduction

Programmed cell death (apoptosis) is an important process in the development and tissue homeostasis. This phenomenon occurs when the cells are exposed to certain toxic agents. Apoptosis is a crucial process for eliminating cancer cells. Therefore, induction-apoptosis is a key mechanism by which anticancer therapy works.[1–3] In recent years, attention has been paid to potential anticancer components from traditional Chinese medicinal herbs that can result in induction of cell apoptosis.[4–6] Some flavones and polyphenols present in traditional Chinese medicines have been demonstrated to decrease various types of experimental carcinogenesis.[7] *Saxifraga stolonifera* (*L*) Meeb (Saxifragaceae), which belongs to dicotyledon, is a perennial herbaceous plant growing at an altitude of 390–3600 m in China, Russia, Japan and Korea. The whole plant is known for its use in Chinese medicines to treat measles, tympanitis, erysipelas, hemoptysis, piles, and hair fall.[8] Pharmacological experiments have indicated that constituents and extracts of *S. stolonifera* can block tumors at various sites, e.g. gastric, prostate, breast and leukemia. Studies have also revealed that the extracts of *S. stolonifera* can inhibit proliferation of cancer cells in vivo by induction of apoptosis.[9,10] It can serve as a prescribed Chinese medicine to treat cholecystitis and viral diseases,[11] and as an effective anti-wrinkle agent on human skin.[12] Although there have been some reports on the isolation of flavones, organic acids, and bergenin from *S. stolonifera* leaves, no attempt has been made to study the bioactivity and identification of active constituents of *S. stolonifera* plant as a whole.[13,14]

In this study we have attempted to evaluate the growth inhibitory and apoptosis inducing activity of *S. stolonifera* in human gastric carcinoma cells BGC-823 with special emphasis on its mode of action. The entire plant *S. stolonifera* collected from Guizhou province was studied and 10 compounds were isolated from the ethanol

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extracts, among which four compounds were isolated from this plant for the first time. We evaluated the anticancer activities of these compounds by MTT assay and microscopic observation, DNA fragmentation, and flow cytometry analysis assay. It was found that quercetin can induce apoptosis in BGC-823 cancer cells.

2. Chemistry

During this investigation, the chemical constituents of the whole plant *S. stolonifera*, grown in Guizhou province, were studied. Ten compounds have been isolated from ethanol extracts and identified as *n*-$C_{31}H_{64}$ (**1**), (*n*-$C_{17}H_{35})_2CO$ (**2**), β-sitosterol (**3**), *n*-$C_{29}H_{60}$ (**4**), Bergenin (**5**), Protocatechuic acid (**6**), Gallic acid (**7**), Quercitrin 3-*O*-α-L-rhamnoside (**8**), Quercetin (**9**), and quercetin 3-*O*-β-D-gluco-pyranoside (**10**) by analytical and spectral methods (Scheme 1). Among them, *n*-$C_{31}H_{64}$ (**1**), (*n*-$C_{17}H_{35})_2CO$ (**2**), β-sitosterol (**3**), and *n*-$C_{29}H_{60}$ (**4**) were isolated from this plant for the first time.

2.1. Identification of compounds

2.1.1. Compound 1: *n*-$C_{31}H_{64}$. White squama crystal; mp 61–62 °C; Mass spectra (MS) indicate that the compound is an *n*-alkane with molecular formula of $C_{31}H_{64}$. The compound was identified further by ^1H NMR and ^{13}C NMR. IR (KBr) cm^{-1}: 2852, 2954, 1462, 1377, 727, 719; MS (*m*/*z*): 435 (M^+-1), 113, 99, 85, 71, 57, 43, 29; and ^1H NMR ($CDCl_3$, 500 MHz): 0.78–0.82 (6H, m, $2CH_3$), 1.22 (58H, s, $29CH_2$); ^{13}C NMR ($CDCl_3$,125 MHz): δ 14.12 (2C, 1, 1′-C), 22.70 (2C, 2, 2′-C), 29.38–31.94 (27C). As analyzed above, compound **1** was identified as *n*-$C_{31}H_{64}$.[15]

2.1.2. Compound 2: (*n*-$C_{17}H_{35})_2CO$. White powder; mp 80.7–83 °C; IR and ^{1}HNMR indicate that the compound is an *n*-dialkyl ketone with symmetrical character. The compound was further identified by ^1H and ^{13}C NMR. IR (KBr) cm^{-1}: 2916, 2846, 1707 (C═O), 1471, 1462, 719; ^1H NMR (500 MHz, $CDCl_3$): 0.88 (6H, t, J = 1.2 Hz, $2CH_3$), 1.25 (56H, s, $28CH_2$), 1.62–1.69 (4H, m, β-H of —C═O), 2.35 (4H, t, J = 2.3 Hz, α-H of —C═O); ^{13}C NMR (125 MHz, $CDCl_3$): 178.72 (C═O), 33.79 (2C, α-C), 31.92 (2C, β-C), 29.69–29.05 (24C), 24.68 (2C, β′-C), 22.69 (*a*′-C), 14.12 (2C, CH_3). As analyzed above, compound **2** was identified as (*n*-$C_{17}H_{35})_2CO$.[16]

2.1.3. Compound 3: β-Sitosterol. White acerate crystal (petroleum-ether); nature of TLC was the same as that of a standard β-sitosterol sample and melting point (137–139 °C) was also consistent with this standard sample. IR spectra indicate that the molecule has —OH and C═C groups; Mass spectra show molecular ion M^+ as 414. The compound was identified as β-sitosterol;[17] IR (KBr) cm^{-1}: 3429, 3373 (—OH), 2959, 2866, 1463, 1367(C═C); MS (*m*/*z*): 414 (M^+), 400, 387 329, 303, 213, 99, 85, 71, 57, 43, 29; ^1H NMR ($CDCl_3$, 500 MHz): 3.52 (1H, s, —OH), 5.35 (1H, s, 7-H); ^{13}C NMR ($CDCl_3$, 125 MHz): 140.7 (C-5), 121.7 (C-7), 71.7(C-1), 56.7 (C-17), 56.0 (C-14), 50.1 (C-21), 45.8 (C-10), 42.3 (C-13), 19.8 (C-29), 19.4 (C-28), 19.0 (C-25), 18.7 (C-26), 11.9 (C-27), 11.8 (C-4).

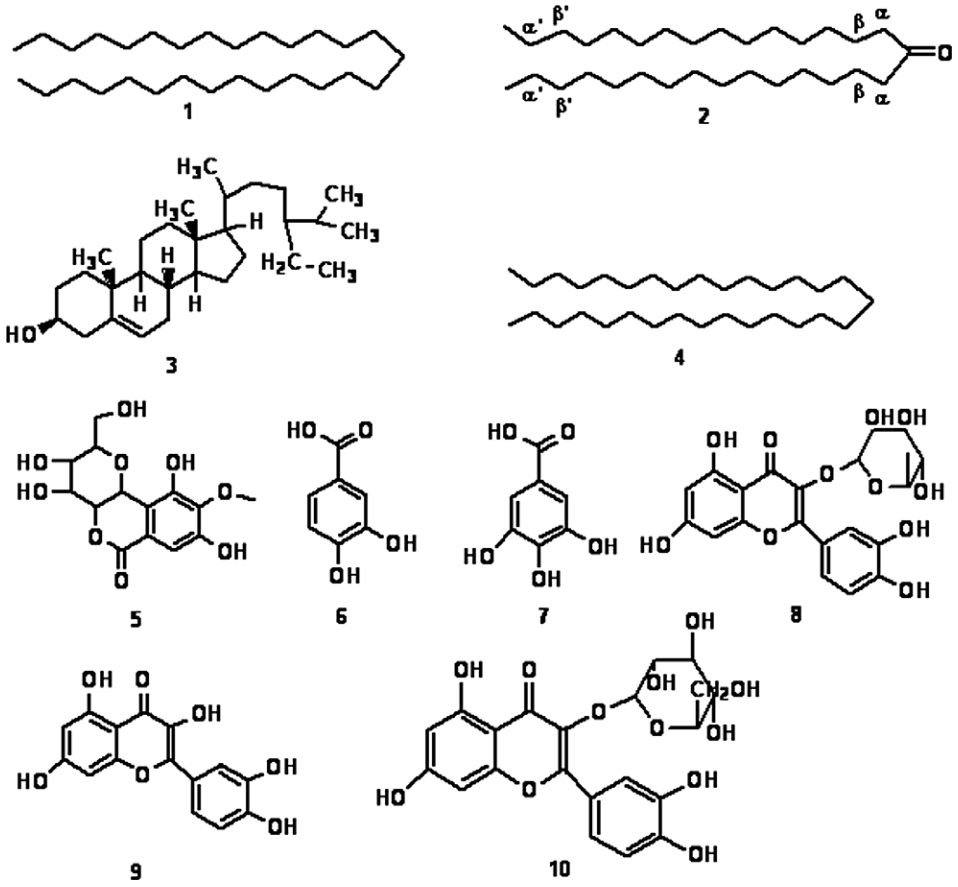
2.1.4. Compound 4: *n*-$C_{29}H_{60}$ (Nonacosane). White squama crystal ($CDCl_3$); mp 58–60 °C; IR and MS indicate that the compound is an *n*-alkane with a molecular formula of $C_{29}H_{60}$. The compound was identified by ^1H NMR and ^{13}C NMR. IR (KBr) cm^{-1}: 2916, 2848, 1471, 1462, 729, 719; MS (*m*/*z*): 408 (M^+): 379, 365, 351, 337, 323, 309, 295, 281, 267, 253, 239, 225, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57, 43, 29; ^1H NMR ($CDCl_3$, 500 MHz): 0.88 (6H, t, J = 4.45 Hz, $2CH_3$), 1.25 (54H, s, $27CH_2$); ^{13}C NMR ($CDCl_3$, 125 MHz): 14.23 (2C, 1, 1′-C), 22.80 (2C, 2,2′-C), 29.47–32.03 (25C). As analyzed above, it was identified as *n*-$C_{29}H_{60}$.[18]

2.1.5. Compound 5: Bergenin. White tabular crystal (CH_3OH), mp 147–150 °C; molecular formula: $C_{14}H_{16}O_9$; it turned green on treatment with ferric chloride solution; IR (KBr) cm^{-1}: 3421, 3387 (—OH), 2893, 2852 (CH_2), 1701 (C═O), 1612, 1527 (aromatic), 1348, 1234 (aromatic C—O), 858 (C—H); EI-MS *m*/*z*: 328 (M^+), 208, 195, 190, 180, 152, 109, 103, 61; ^1H NMR (CD_3OD, 500 MHz): 3.66–3.74 (2H, m, 13-H), 3.79 (1H, s, 12-H), 3.81 (3H, t, J = 7.5 Hz, 14-H), 4.02 (2H, d, J = 5.8 Hz, 10, 11-H), 4.87 (5H, s, —OH), 4.92 (2H, d, J = 4.2 Hz, 2, 3-H), 7.05 (1H, s, 8-H); ^{13}C NMR (CD_3OD, 125 MHz): 164 (C-1), 151 (C-7), 148 (C-5), 141 (C-6), 118 (C-9), 116 (C-4), 109 (C-8), 106 (C-2), 80 (C-3), 74.3 (C-10), 72.9 (C-11), 70.5 (C-12), 61.3 (C-13), 59.6 (C-14); DEPT indicates the presence of CH_2 group. As analyzed above, compound **5** is identified as Bergenin.[19,20]

2.1.6. Compound 6: Protocatechuic acid. White acerate crystal (H_2O); mp 194–196 °C; it turned ferric chloride solution blue; molecular formula: $C_7H_6O_4$; IR (KBr) cm^{-1}: 3600 (—OH), 1676, 1600 (C═O); MS (*m*/*z*): 154 (M^+), 137, 109. Compound **6** was identified as Protocatechuic acid.[21]

2.1.7. Compound 7: Gallic acid. White acerate crystal (CH_3OH); mp 250–252 °C; it turned ferric chloride solution blue; Molecular formula: $C_7H_6O_5$; IR (KBr) cm^{-1}: 2661–3493 (—OH), 1691(C═O), 1612, 1541, 1431 (aromatic); MS (*m*/*z*): 170 (M^+), 153, 125; ^{1}HNMR (Me_2CO-d_6, 500 MHz): 7.15 (s, 2H, 2, 6-H); ^{13}C NMR (Me_2CO-d_6, 125 MHz): 167.2 (—CO), 145.0 (C-3, 5), 137.7 (C-4), 121.0 (C-1), 109.1 (C-2, 6). As analyzed above, compound **7** was identified as Gallic acid.[22]

2.1.8. Compound 8: Quercitrin 3-*O*-α-L-rhamnoside. Yellow powder (CH_3OH); mp 182–184 °C; it turned ferric chloride solution green; Molecular formula: $C_{21}H_{20}O_{11}$; IR (KBr) cm^{-1}: 3523 (—OH), 1658 (C═O), 1604, 1566, 504, 1444 (aromatic), 1365, 1058, 815; ^1H NMR (DMSO-d_6, 500 MHz): 0.80 (3H, d, J = 5.3 Hz, H-6″), 3.11–3.20 (1H, m, H-4″), 3.15–3.23 (1H, m, H-3″), 3.51–3.62 (1H, m, H-2″), 3.91–4.01 (1H, m, H-5″), 5.25 (1H, s, H-1″), 6.20 (1H, d, J = 6.3 Hz, H-6), 6.39 (1H, d, J = 3.7 Hz, H-8), 6.86 (1H, d, J = 9.3 Hz, H-5′),



Scheme 1. The structure of compounds **1**–**10**.

7.25 (1H, dd, J = 5.3, 1.3 Hz, H-2′), 7.30 (1H, d, J = 2.4 Hz, H-6′), 12.66 (1H, s, OH); ^{13}C NMR (CD_3OD, 125 MHz): 179.6(C-4), 165.8 (C-7), 163.2 (C-5), 159.3 (C-9), 158.5 (C-2), 149.8 (C-3′), 146.4 (C-4′), 136.2 (C-3), 122.9 (C-1′), 122.8 (C-6′), 116.9 (C-5′), 116.3 (C-2′), 105.9 (C-10), 103.5 (C-6), 99.8 (C-8), 94.7 (C-1″), 73.2 (C-4″), 72.1 (C-2″), 72.0 (C-3″), 71.9 (C-5″), 17.6 (C-6″). As analyzed above, compound **8** was identified as Quercitrin 3-*O*-α-L-rhamnoside.[23,24]

2.1.9. Compound 9: Quercetin. Yellow acerate crystal (CH_3OH); mp 314–316 °C, turned green on treatment with ferric chloride; molecular formula: $C_{15}H_{10}O_7$; IR (KBr) cm^{-1}: 3500 (OH), 1662 (C═O), 1614, 1512 (aromatic); MS (*m*/*z*): 302 (M^+), 286, 273, 258, 245, 229, 215, 200, 171, 153, 137, 109, 69; ^{1}H NMR (CD_3OD, 500 MHz): 6.18 (1H, d, J = 1.7 Hz, H-6), 6.38 (1H, d, J = 2. 4Hz, H-8), 6.88 (1H, d, J = 8.6 Hz, H-5′), 7.63 (1H, dd, J = 8.5, 3.2 Hz, H-6′), 7.73 (1H, d, J = 2.3 Hz, H-2′); ^{13}C NMR (CD_3OD, 125 MHz): 175.9 (C-4), 164.2 (C-7), 161.2 (C-5), 156.8 (C-9), 147.4 (C-2), 146.6 (C-3′), 144.9 (C-4′), 135.9 (C-3), 122.8 (C-1′), 120.3 (C-6′), 114.9 (C-5′), 114.6 (C-2′), 103.2 (C-10), 97.9 (C-6), 93.0 (C-8). As analyzed above, compound **9** was identified as Quercetin.[25,26]

2.1.10. Compound 10: Quercetin 3-*O*-β-D-glucopyranoside. Yellow powdery crystal (CH_3OH); mp 228–230 °C; Molecular formula: $C_{21}H_{20}O_{12}$; IR (KBr) cm^{-1}: 3600 (—OH), 1654 (C═O), 1606, 1556, 504, 1504 (aromatic), 1363, 1203, 1089; ^{1}H NMR (500 MHz, CD_3OD) δ: 3.46–3.84 (6H, m, Glu-H), 5.15 (1H, d, J = 8.1 Hz, H-1″), 6.18 (1H, d, J = 1.8 Hz, H-6), 6.38 (1H, d, J = 1.7 Hz, H-8), 6.84 (1H, d, J = 8.6 Hz, H-5′), 7.75 (1H, dd, J = 6.3, 2.3 Hz, H-6′), 7.82 (1H, d, J = 2.3 Hz, H-2′); ^{13}C NMR (CD_3OD, 125 MHz) δ: 178.2 (C-4), 164.7 (C-7), 161.7 (C-9), 157.5 (C-5), 157.1 (C-2), 148.6 (C-4′), 144.5 (C-3′), 134.4 (C-3), 121.6 (C-1′), 121.5 (C-6′), 116.4 (C-5′), 114.7 (C-2′), 104.3 (C-10), 98.5 (C-8), 93.4 (C-6), glucopyranoside C1″ → C6″, 104.1 (C-1″),

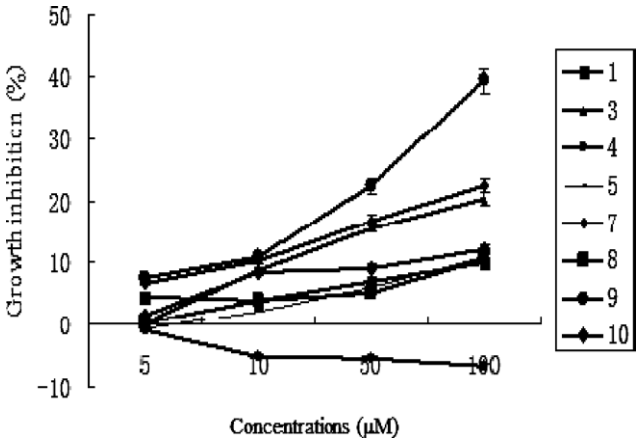


Figure 1. Growth inhibition effect of constituents of *Saxifraga stolonifera* on BGC-823 cells. Each value represents the mean ± SD (n = 8).

Table 1. Growth inhibition effects of different constituents of *Saxifraga stolonifera* on BGC-823 cells[a]

Compound	Growth inhibition (%)			
	5 μM	10 μM	50 μM	100 μM
n-$C_{31}H_{64}$	4.5 ± 0.02	3.9 ± 0.06	7.0 ± 0.05	10.1 ± 0.04
β-Sitosterol	0.4 ± 0.01	8.8 ± 0. 02	15.6 ± 0.02	20.4 ± 0.04
n-$C_{29}H_{60}$	−0.9 ± 0.02	−5.2 ± 0.02	−5.6 ± 0.02	−6.6 ± 0.03
Bergenin	−0.8 ± 0.02	2.2 ± 0.03	6.1 ± 0.02	10.8 ± 0.02
Gallic acid	6.7 ± 0.02	10.2 ± 0.05	16.7 ± 0.02	22.5 ± 0.03
Quercitrin 3-*O*-α-L-rhamnoside	0.2 ± 0.02	3.8 ± 0.03	5.2 ± 0.02	10.6 ± 0.02
Quercetin	7.5 ± 0.01	11.0 ± 0.01	22.4 ± 0.01	39.3 ± 0.02
Quercetin 3-*O*-β-D-glucopyranoside	1.5 ± 0.01	8.4 ± 0.02	9.2 ± 0.01	12.1 ± 0.03

[a] Each value represents average of eight independent samples. BGC-823 cells were plated into 96-well plates for 24 h and were then treated with four concentrations (5, 10, 50, and 100 μM) of different constituents from *S. stolonifera* for another 72 h. MTT was added to the medium for an additional 4 h with concentration of 1 mg/mL. The viability of cells was detected by measuring the absorbance at 490 nm.

75.9 (C-3″), 73.8 (C-2″), 71.8 (C-4″), 68.7 (C-5″), 60.6 (C-6″). As analyzed above, compound **10** was identified as quercetin 3-*O*-β-D-gluco-pyranoside.[27]

3. Anticancer activity

3.1. Cell viability affected by constituents from *S. stolonifera*

As shown in Figure 1 and Table 1, the growth inhibitory effect of the extracts from *S. stolonifera* was studied on human tumor cell lines BGC-823 by MTT assay at concentrations ranging from 5 to 100 μM. It could be seen that the inhibitory effects of β-sitosterol, gallic acid and quercetin were concentration-dependent. Among these ingredients, quercetin was found to exhibit high effect on BGC-823 cells, with the growth inhibition ratio of 39.3% after 72 h treatment at 100 μM, while the growth inhibition ratios of other compounds were considerably lower even at high concentration, ranging from −6.6% to 22.5% after 72 h treatment at 100 μM. It was noticed that quercetin not only caused growth inhibition in BGC-823 cells, but also brought out morphological changes on the tumor cells. The results are shown in Figure 2, where 'A' denotes the control tumor cells (untreated with quercetin), which are small, closely packed, and polygonal. After being exposed to quercetin for 24 h, chromosomal condensations were induced (Fig. 2C). When the tumor cells were treated by quercetin for 48 h, it induced the formation of apoptotic bodies in (D).

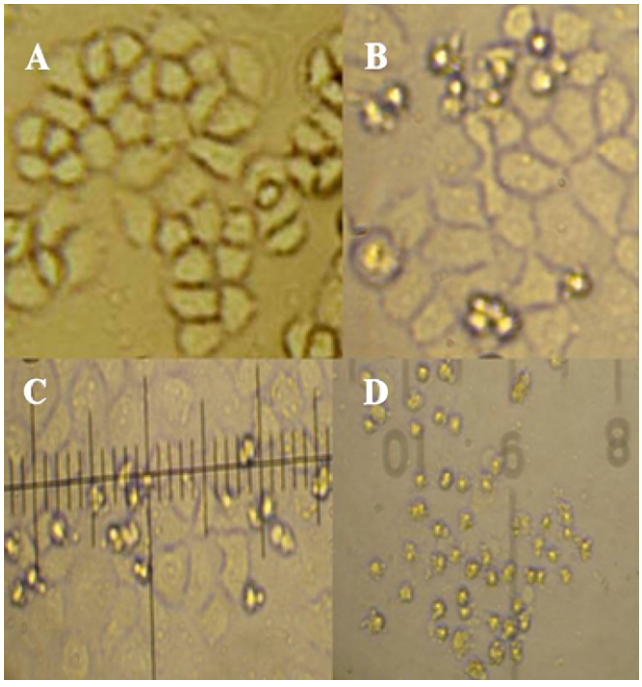


Figure 2. Appearance of chromosomal condensation and apoptotic bodies in quercetin-treated BGC-823 cells under microscopic observation. Letters A, B, C and D in the figures represent treatment time of the cells (0, 12, 24, 48 h) by quercetin at 50 μM.

3.2. Induction of apoptosis by compound 9 (quercetin)

3.2.1. DNA electrophoresis. To characterize cells apoptosis induced by quercetin, the DNA ladder was examined. As shown in Figure 3, quercetin caused the digestion of

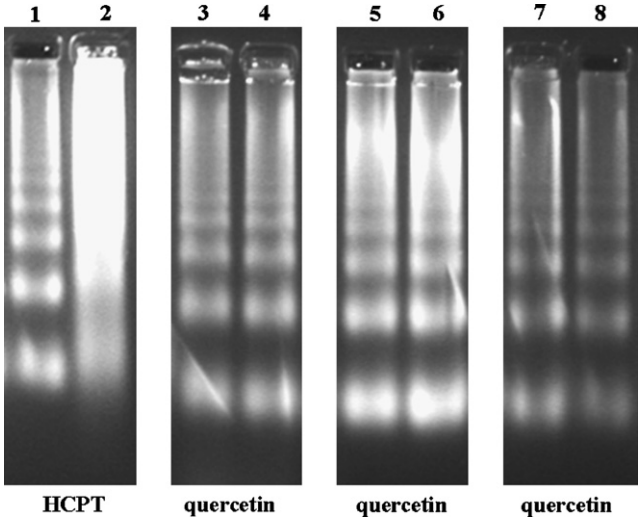


Figure 3. Agarose gel electrophoresis of DNA extracted from BGC-823 cells. The figure represents that BGC-823 cells were treated with quercetin and HCPT. DNA in cells was extracted and electrophoresed through a 1.2% agarose gel and visualized by staining with ethidium bromide. Lane 1, positive control: treated by HCPT (20 μM) for 36 h; lane 2, negative control (without treatment); lane 3, treated by quercetin (50 μM) for 12 h; lane 4, treated by quercetin (100 μM) for 12 h; lane 5, treated by quercetin (50 μM) for 24 h; lane 6, treated by quercetin (100 μM) for 24 h; lane 7, treated by quercetin (50 μM) for 48 h; lane 8, treated by quercetin (100 μM) for 48 h.

genomic DNA into ladders in concentration- and time-dependent ways in BGC-823 cells. After exposure of quercetin for 12 h, DNA fragmentation in 180 bp was detected in the cells. When BGC-823 cells were treated with quercetin for 48 h at 50 and 100 μM, a remarkable increase in DNA fragmentation was observed. However, with other compounds, even at 100 μM, no significant amount of DNA ladders was found in BGC-823 cells (data not shown). Meanwhile, positive control (treated with 20 μM of Hydroxycamptotecin (HCPT) for 36 h) and negative control (without treatment) were both set in the DNA ladder assay (Fig. 3, lanes 1 and 2, respectively).

3.2.2. Flow cytometric analysis. BGC-823 cells (2×10^6) were incubated with quercetin at 100 μM for 0–48 h at 37 °C. Cells were collected and the induction apoptosis was studied. As shown in Figures 4 and 5, quercetin can induce apoptosis of BGC-823 cells. When BGC-823 cells were treated with quercetin, the apoptosis ratio kept increasing until it reached the peak (11.82%) at 24 h, after which it dropped slightly. Figures 4 and 5 show that the apoptosis ratios of BGC-823 cells treated with quercetin and HCPT at different time were 0.29%, 5.02%, 11.82%, 10.19%, 10.23%; 0.18%, 3.72%, 4.78%, 13.2%, 6.47%.

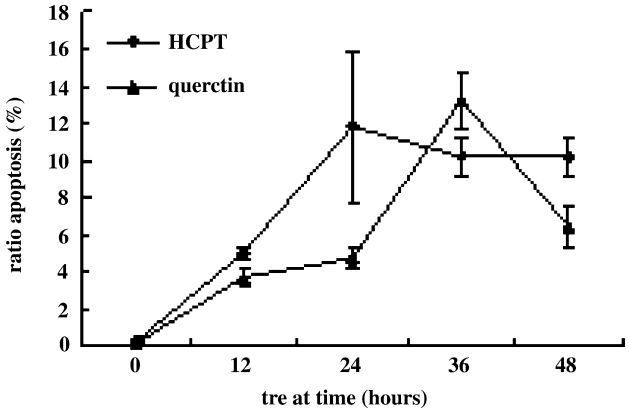


Figure 4. The ratio of apoptosis of BGC-823 cells after being treated with 100 μM quercetin and 20 μM HCPT for 0–48 h. Data presented are means ± SD of three independent experiments. Mean values of three replicates were analyzed for interactive line in SPSS 10.0.

4. Conclusions

We isolated and identified 10 compounds from *S. stolonifera* and studied their inhibitory effect on the tumor cells BGC-823 by MTT assay.

Quercetin was found to exhibit high inhibitory effect on BGC-823 cells in a time- and dose-dependent manner. The growth inhibition ratio of quercetin on BGC-823 cells was 39.3% after 72 h treatment at 100 μM. The other compounds did not show significant growth inhibition activity.

Although there are reports concerning induction apoptosis by quercetin on human promyelocytic leukemia cells (HL-60 cells) and kidney tubule epithelial cells (NRK-52E),[1,2] similar reports are not available on BGC-823 cells. We evaluated the induced-apoptosis and the apoptosis ratio by DNA ladder and flow cytometric analysis and found that quercetin can induce apoptosis on BGC-823 cells. Furthermore, DNA ladder assay revealed that quercetin-induced DNA strand breaks at 180 bp in a concentration- and time-dependent fashion. Flow cytometric analysis showed that quercetin can induce 11.8% apoptosis of BGC-823 cells in 48 h. Phenomenon of induction-apoptosis was not noticed with other compounds by morphological observation, DNA ladder assay,and flow cytometric analysis. Quercetin seems to hold promise for inducing apoptosis in BGC-823 cells. The data indicate that quercetin from *S. stolonifera* has the potential to be used as a chemopreventive and therapeutic agent for cancers.

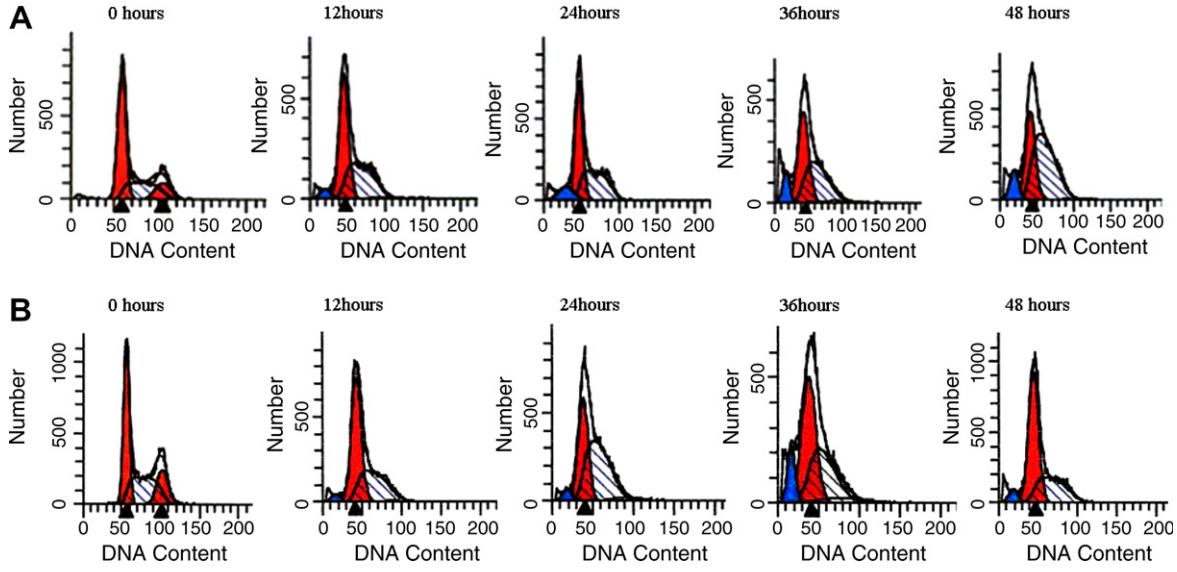


Figure 5. Flow cytometry analysis for apoptosis of BGC-823 cells and effects of induction aopotosis by quercetin (A) and HCPT (B) on the cells. BGC-823 cells were incubated with 100 μM quercetin and 20 μM HCPT for 0–48 h. The appearance of apoptosis cells was detected by flow cytometry using PI staining. The DNA PI staining was measured by flow cytometry.

5. Experimental

5.1. Analysis and instruments

Melting points were determined on an XT-4 binocular microscope (Beijing Tech Instrument Co.); IR spectra were obtained in KBr disks flake on SHIMADZU-IR Prestige-21 spectrometer. MS data were obtained on a HPMS 5973 spectrometer. ^{1}H NMR, ^{13}C NMR, and DEPT spectra were recorded in $CDCl_3$, CD_3OD, and DMSO on a at JOEL-ECX 500MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Column chromatography procedures (CC) were performed with Silica gel (100–200 or 200–300 mesh, Qingdao Haiyang Chemical Co.) and polymeric amide (100–200 mesh, Zhejiang Taizhou Tuqiao Biochemical Plastic Co.).

5.2. Plant material

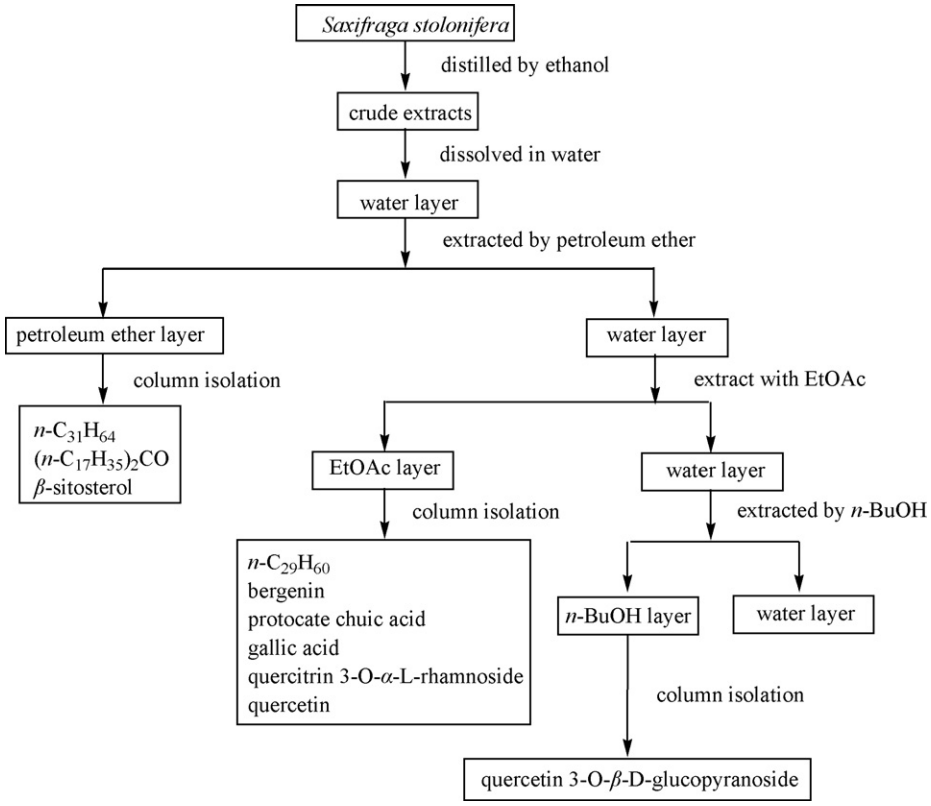
The whole plant *S. stolonifera* (L) Meeb was collected in Guiyang, Guizhou province, China, in June 2005. The plants were identified as *S. stolonifera* (L) Meeb by druggist Huang, Huaxi yuansheng Big Pharmacy (Huaxi Guiyang Guizhou). A voucher specimen was deposited in our laboratory.

5.3. Extraction and isolation

Dried whole plants (4 kg) of *S. stolonifera* were extracted with ethanol (each 20 L) three times (each 2 h) at boiling temperature. The ethanol extract was concentrated, suspended in water, and sequentially partitioned with petroleum ether, EtOAc, and *n*-BuOH assisted by ultrasound.

The petroleum ether extract (60.0 g) was absorbed onto silica gel (60.0 g) and then chromatographed on a silica gel (600 g) column eluted with petroleum ether–EtOAc (1:0, 15:1,10:1, 8:1, 5:1, 3:1, 2:1, 1:1, 0:1) gradients. The eluted fractions were evaluated by TLC and combined to give fractions 1–9, respectively. Fractions 1, 5, and 8 were recrystallized from $CHCl_3$ to afford compounds **1** (80 mg), **2** (65 mg), and **3** (300 mg), respectively.

The EtOAc extract (70.0 g) was absorbed onto silica gel (75.0 g) and then chromatographed on a silica gel (1500 g) column eluted with petroleum ether–acetone (1:0, 30:1, 20:1, 15:1, 10;1, 6:1, 4:1, 2:1, 0:1) gradients. The eluted fractions were evaluated by TLC and combined to give fractions 1–5, respectively. Fractions 1 and 5 were recrystallized from $CHCl_3$ and EtOH, respectively, to afford compounds **4** (400 mg) and **5** (2.5 g). The filtrate from fraction 5 (34.0 g) was absorbed onto silica gel (30.0 g) and then chromatographed on a silica gel (400 g) column eluted with $CHCl_3$-acetone (1:0, 10:1, 5:1, 1:1, 0:1) gradients. The eluted fractions were evaluated by TLC and combined to give subfractions 1–8, respectively. Subfractions 2, 4, and 6 were separated on polymeric amide (each 20 g) column, respectively, with H_2O–ethanol (1:0, 4:1, 3:2, 2:3) to afford pure compounds **6** (20 mg), **7**



Scheme 2. The isolation process for different constituents from *Saxifraga stolonifera*.

(100 mg), and **8** (250 mg); subfraction 7 was separated on polymeric amide (20 g) column with H_2O-ethanol (1:0, 4:1, 3:2, 2:3) to afford pure compound **9** (120 mg).

The *n*-BuOH extract (50.0 g) was fractionated by a combination of silica gel and polymeric amide column chromatography to afford compound **10** (120 mg). The isolation process for different constituents from *Saxifraga stolonifera* is shown in Scheme 2.

5.4. Anticancer activity bioassay

BGC-823 cells were purchased from Institute of Biochemistry and Cell Biology, China Academy of Science. The tumor cells were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) in CO_2 incubators at 37 °C in 5% CO_2 and 95% air. Cancer cells were exposed to compounds at indicated concentration for different time periods. The plate and cell culture flask for cancer cells and MTT were purchased from Shanghai Sangon Biotechnology Company, Ltd. Agarose gel was purchased from Sino-American Biotechnology Company. HCPT was obtained from Shenzhen Main Luck Pharmaceuticals Inc.

5.4.1. Cell viability. Cell viability was assessed by MTT assay as described by Mosmann.[28] Briefly, BGC-823 cells were plated at a density of 2×10^4 cells/mL into 96-well plates and treated with 100 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (1 mg/mL). The cells were incubated for a further 4 h. The formazan crystals formed were dissolved in DMSO (dimethyl sulfoxide). The extent of MTT reduction was measured by using an ELISA reader (Bio-Rad Model 680 Microplate Reader) with a 490-nm filter. Growth inhibition was obtained as percentage over the untreated control.

5.4.2. DNA fragmentation (DNA ladder) assay. DNA ladder was assessed by DNA fragmentation assay as described by Daniel.[29] Briefly, BGC-823 cells were plated at a density of 3×10^4 cells/mL into 60 mm culture dish. The cells were treated by quercetin at 50 and 100 μM concentrations and DNA samples were extracted from cells at 12, 24, and 48 h. Cells from different treatment groups were collected separately. Cells were washed once with ice–PBS, centrifuged, and the supernatants were removed carefully. The cells were dispersed in 50 μl of lysis buffer (10 mM Tris, pH 7.4; 100 mM NaCl; 25 mM EDTA; 1% sarkosyl) and incubated at 50 °C for 3 h. Four microliters of DNA sample dye was mixed with the bromophenol blue buffer, and then electrophoresed on 1.2% agarose gel at 50 V for 3 h. The electrophoresis was stopped when bromophenol blue dye reached the foreland of the gel and the DNA was visualized under UV light.

5.4.3. Flow cytometry analysis. Trypsinized cells (1×10^6/mL) were washed twice in ice-cold Hanks and fixed in 75% ice-cold ethanol for at least 1 h. After fixation, cells were washed twice with PBS, then stained in 100 μg/mL PI (Sigma), 10 μg/mL Rnase (Sigma) in PBS, and incubated for 30 min at 37 °C. Fluorescence emitted from the propidium–DNA complex was quantitated after excitation of the fluorescent dye by FACS Calibur, Becton–Dickinson.

5.4.4. Statistics. Values are expressed as means ± SD. The significance of the difference between control and each treatment was evaluated using the Student's *t*-test, and difference was considered significant at $P < 0.05$.

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