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Original Research Article

Antioxidant and Anti-proliferative Activities of Flavonoids from *Bidens pilosa* L var *radiata* Sch Bip

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Abstract

Purpose: *To investigate the chemical composition of Bidens pilosa L. var. radiata. Sch Bip. (BP), as well as its antioxidant and anti-proliferative activities.*
Methods: *The whole herb of BP was extracted with 95 % ethanol, which was then partitioned sequentially with petroleum ether, ethyl acetate and n-butyl alcohol to obtain petroleum ether, ethyl acetate (EE-BP), n-BuOH and water fractions. EE-BP was further isolated and purified by repeated column chromatography. The obtained compounds were identified by comparing ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra with published data for the compounds. Flavonoids were evaluated for 1,1-diphenyl-2-picrylhydrazy (DPPH) and 2, 2 -azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity by colorimetric methods, while their anti-proliferative effect against human colon cancer RKO cells was determined by 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) method.*
Results: *A total of 6 compounds were isolated from BP, including flavonoids or flavonoid glycosides, among which were isoquercitrin (1), vitexin (2), astragalin (3), 5,6,7,4'-tetramethoxylflavone (4), 5,3',4'-trihydroxy-3,7-dimethoxylflavone (5) and quercetin (6). The results indicate that compound 6 was the most effective in both DPPH and ABTS free radical-scavenging assays, with half-maximal inhibitory concentration (IC_{50}) of 10.7 µmol/L and 17.5 µmol/L, respectively. Both compounds 4 and 5 exhibited significant growth inhibitory effect on RKO cells with IC_{50} of 39.08 µmol/L and 17.68 µmol/L, respectively ($p < 0.01$).*
Conclusion: *BP contains a series of flavonoid compounds, possessing significant antioxidant activity and antiproliferative effect in RKO cells, thus suggesting that it is a potential health supplement and an easily available resource of natural antioxidants for use in colon cancer prevention.*

Keywords: *Bidens pilosa, Flavonoid, Antioxidant, Anti-proliferation, Colon cancer*

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INTRODUCTION

Mounting evidence has shown that overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxide anion and hydroxyl radicals, hydrogen peroxide, nitric oxide, nitrosonium, nitroxyl anion and peroxynitrite, can cause DNA damage resulting in cell malfunction [1], and this ultimately leads to cancer due accumulation of somatic mutations [2]. Furthermore, uncontrolled cell proliferation would eventually result in a net increase in the number of cancer cells, which is strongly associated with the development and progression of cancer [3]. In this respect, removing ROS/RNS generated in excess and

restraining cancer cell proliferation are considered an effective means to fight cancer [4]. Recently, a great concern has been focused on searching for potential anticancer agents of natural origin [5]. Flavonoids, ubiquitously distributed in plants, are plant-derived polyphenolic molecules that are well known as effective antioxidants, preventing oxidative stress induced by ROS/RNS [6]. Moreover, the growing epidemiologic evidence has demonstrated that flavonoid consumption is associated with a decreased risk of colorectal cancer, which has recently been documented by a large case-control study [7]. Hitherto, a number of flavonoids, exemplified by isoquercitrin [8], quercetin [9], and catechin [10] have been shown to exert anti-colorectal cancer activity by inhibiting cell proliferation.

Bidens pilosa Linn. var. *radiata* Sch. Bip., belonging to Asteraceae family, originated from South America and can be found in almost all countries from tropical and subtropical regions [11]. And there are three common variants of this species: *Bidens pilosa* Linn. var. *radiata* Sch. Bip., *Biden pilosa* L. var. *pilosa* and *Bidens pilosa* L. var. *minor* [12]. They are usually employed in traditional medicines as a remedy to treat various diseases, such as hepatitis, stomach disorders, diabetes, hypertension, malaria, inflammation and digestive disorders [13]. Although *Bidens* species have been studied a lot to our best knowledge, the chemical constituents and their bioactivities from this variant remain largely unknown.

Recently, several compounds have been first isolated from this plant in our previous work, including flavonoids, flavonoid glycosides, phenols and phenylpropanoids [14]. Moreover, 3, 3′-dimethylquercetin was documented to exhibit significant cytotoxicity and apoptosis-inducing activity against RKO cells [15]. As part of our ongoing research on chemical constituents, a total of 6 compounds were successively obtained, mainly flavonoids or flavonoid glycosides. They were further evaluated for the antioxidant activity by using two complementary systems, namely DPPH and ABTS free radical scavenging assays, as well as their growth inhibitory effect against human colon cancer cell lines RKO by the MTT method.

EXPERIMENTAL

Chemicals and reagents

Methanol, ethanol, petroleum ether, ethyl acetate, n-butylalcohol and potassium persulfate were purchased from Sinopharm Chemical Reagent, Co., Ltd. (Shanghai, China). 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), trypsin, and penicillin–streptomycin were obtained from Hyclone Laboratories, INC. (Logan, UT, US). The normal phase silica gel (200-300 mesh) was obtained from Qingdao Marine Chemical Co., China. Sephadex LH-20 (20-100 μm) was obtained from Pharmacia Fine Chemical Co., Ltd, Uppsala, Sweden and RP-18 silica gel was from Merck Co., Germany. Spectrophotometric measurements were performed on an Ultraviolet–visible spectrometer (Shimadzu UV-2008; Japan). All other chemicals were of analytical grade.

Plant material

The whole plant of BP was collected in Fujian province, China, in August 2009 and authenticated by Associate Professor Chengzi Yang, a taxonomist at the Department of Pharmacy, and a voucher specimen (no. 2009-BP) was deposited in the herbarium of Academy of Integrative Medicine, Fujian University of Traditional Chinese Medicine, Fuzhou, China.

Extraction and isolation

The whole plant material (9.0 kg) was dried at room temperature, powdered, and refluxed with 75 % ethanol for 2 × 3 h to obtain total extract (1033.0 g). The alcohol was removed under vacuum using a rotary evaporator. The residue was suspended in water, and partitioned sequentially with petroleum ether, EtOAc and n-BuOH to obtain a petroleum ether fraction, ethyl acetate fraction (EE-BP), n-BuOH fraction and the remainder, the water fraction. EE-BP was evaporated *in vacuo*. EE-BP (90.0 g) was subjected to column chromatography over silica gel (200–300 mesh) developed with petroleum ether–acetone (50:1–1:1). The flavonoid compounds were isolated and purified from the petroleum ether–acetone (3:1) fraction by repeated column chromatography including silica gel, Sephadex LH-20 and ODS-C18 column. TLC was detected with 10 % H_2SO_4-EtOH solution. Melting points were determined on X-4 apparatus and uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with TMS as internal standard on a Bruker AvanceTM 400 MHz. ESI-

MS were recorded on a Bruker Apex Ⅱ micro mass spectrometer.

Determination of DPPH radical scavenging activity

The antioxidant activity of the six compounds was measured on the basis of the scavenging activities of stable DPPH free radical [16]. The values of IC_{50}, concentration of sample required to scavenge 50 % of free radicals were calculated from the regression equation, and percentage inhibition of free radical formation was assayed. BHT was used as positive control. All tests were carried out in triplicate. DPPH radical-scavenging activity (D) was calculated as in Eq 1.

D (%) = {(Ac – As)/Ac}100 …… (1)

where Ac and As are the absorbance of control and test samples, respectively.

ABTS radical scavenging assay

The radical scavenging activity of the six compounds against ABTS radical cation was measured using the method of Re *et al* [17] with some modifications. ABTS was dissolved in water to a concentration of 7 mmol/L. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS radical cation solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 °C. An aliquot of each sample (0.1 mL) was mixed with 3.9 mL of diluted ABTS radical cation solution. The absorbance at 734 nm of the mixture was measured after reaction for 6 min. The ability of individual compound to scavenge ABTS radicals (%) and the IC_{50} were calculated as described in the DPPH radical scavenging assay. BHT was used as positive control.

MTT assay

The human colon cancer RKO cell lines, used for the antitumor activity assay, were obtained from the American type culture collection (ATCC, Manassas, VA, USA), and they were cultured in DMEM supplemented with 10 % FBS, 100 units/ml penicillin, and 100 µg/mL streptomycin in a 37 °C humidified incubator with 5 % CO_2 atmosphere. The anti-proliferative effect of isolated compounds was measured using the standard MTT assay [18]. In brief, exponentially growing cells (about 1 × 10^5 cells/well) were plated into 96-well plates and allowed to adhere for 24 h prior to extract addition. The extract was dissolved in 0.1 % DMSO then diluted with the medium and filtered using 0.22 µm syringe filters. The cells were then exposed to different concentrations of extract. The cells in the control wells received medium containing the same volume of DMSO (0.1 %). After the incubation, 20 µL of MTT reagent (5 mg/mL in PBS) was added and cells were incubated for an additional 4 h. The formazan produced by the viable cells was solubilized by addition of 100 µL DMSO. The suspension was placed on a micro-vibrator for 10 - 15 min and absorbance was recorded at 570 nm with the ELISA reader (BioTek ELx800, Winooski, VT, USA). The experiment was performed in triplicate. Growth inhibition (G) was calculated with respect to vehicle control as in Eq 2.

G (%) = {(Ac – At)/(Ac – Ab)}100 …… (2)

where Ac, At and Ab are the absorbance of control and test and blank samples, respectively.

Statistical analysis

All data are presented as mean ± standard deviation (SD, n = 3). Statistical comparison was performed via one-way analysis of variance followed by Dunnett's test using SPSS Software version18.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Isolation and identification of compounds

By using various column chromatography purification methods, a total of 6 flavonoid compounds were eventually isolated and purified (Fig. 1). They are flavonoids or flavonoid glycosides, identified and listed as follows:

(1) **Isoquercitrin**, $C_{21}H_{20}O_{12}$, yellow needle crystal. mp: 225～227 °C, ESI-MS m/z 463.0 [M-H]-, ^{1}H NMR (400 MHz, DMSO-d6, δ, ppm, J/Hz). d: 12.65 (1H, s , 5-OH), 9.35 (2H, brs, 2×OH) , 7.56 (2H, d, J=8.1, H-2' and H-6'), 6.81 (1H, dd, J=3.6, J=8.5, H-5'), 6.41 (1H, d, J=1.6, H-8), 6.21 (1H, d, J=2.1, H-6), 5.46 (1H, d, J=7.2, H-1''); 13C-NMR (100 MHz, DMSO-d6, δ, J/Hz): 177.86 (C-4), 164.66 (C-7), 161.69(C-5), 156.78 (C-9), 156.61 (C-2), 148.92 (C-4'), 145.27 (C-3'), 133.75 (C-3), 122.06(C-1'), 121.61 (C-6'), 116.64 (C-5'), 115.66 (C-2'), 104.40 (C-10), 101.30 (C-6), 93.97(C-8), 78.03 (C-3''), 76.95 (C-3''), 74.54 (C-2''), 70.38 (C-4''), 61.21 (C-6'') [19].

(2) **Vitexin**, $C_{21}H_{20}O_{10}$, yellow needle crystal. mp: 258～259 °C, ESI-MS m/z 433[M+H]+; ^{1}H NMR (400 MHz, MeOD, δ, ppm, J/Hz): d: 7.61 (1H, d, J=8.0 Hz, H-6'), 7.54 (1H, s, H-2'), 6. 85 (1H, d, J = 8.1 Hz, H-5'), 6.71 (1H, s, H-3), 6.30 (1H, s, H-6), 4.62 (1H, d, J = 8.6 Hz, H-1''); 13C NMR (100 MHz, MeOD, δ, J/Hz): 182.0 (C-4), 167. 2 (C-2), 162. 7 (C-7), 161.2 (C-5), 160.2 (C-4'), 156.1 (C-9), 132.3 (C-2'), 132.0 (C-6'), 120.5 (C-1'), 114.9 (C-3'), 114.3 (C-5'), 104.3 (C-8), 103.5 (C-10) , 102.3 (C-3), 99.2 (C-6), 84.9 (C-5''), 78.9 (C-3''), 78.2 (C-1''), 70.9 (C-2''), 70.0 (C-4''), 60. 8 (C-6'') [20].

(3) **Astragalin**, $C_{21}H_{20}O_{11}$, yellow powder. mp: 163～165 °C, ESI-MS m/z 449 [M+H]+; ^{1}H NMR (500 MHz, DMSO-d6, δ, ppm, J/Hz) d: 12.39 (1H, s, -OH), 10.94 (1H, s, -OH), 10.35 (1H, s, -OH),8.05 (2H, d, J=8.0 Hz, H-2' and H-6'), 6.89 (2H, d, J=8.0 Hz, H-3' and H-5'), 6.45 (1H, s, H-8), 6.22 (1H, s, H-6), 5.45 (1H, d, J=7.5 Hz, H-1"), 5.32~3.09 (10H, H-2"~6", 4-OH). 13C NMR (DMSO-d 6, 125 MHz) δ, ppm, 177.8 (C-4), 164.1 (C-7), 161.4 (C-5), 159.5 (C-9), 156.3 (C-4'), 156.2 (C-2), 133.22(C-3), 130.8 (C-2' and C-6'), 120.2 (C-1'), 115.1 (C-3' and C-5'), 104.3 (C-1"), 100.9 (C-10), 98.9 (C-6), 93.5 (C-8), 77.4 (C-2"), 76.4 (C-5"), 74.3 (C-4"), 69.2 (C-3"), 60.6 (C-6") [21].

(4) **5,6,7,4'-tetramethoxylflavone**, $C_{19}H_{18}O_6$, yellow powder. mp: 142～143 °C, ^{1}H NMR (400 MHz, MeOD, d, ppm, J/Hz). d: 7.95 (2H, dd, J=8.1, 2.1 Hz, H-6' and H-2'), 7.12(2H, dd, J=8.1, 2.3 Hz, H-5' and H-3'), 7.06 (1H,s, H-3), 6.62 (1H, s, H-8), 3.92 (3H, s, 5- OCH3), 3.78 (3H, s, 7- OCH3), 3.41 (3H, s, 6-OCH3), 3.01 (3H, s, 4'-OCH3) [22].

(5) **5,3',4'-trihydroxy-3,7-dimethoxylflavone**, $C_{17}H_{14}O_7$, yellow powder. mp: 218～220 °C, ^{1}H NMR (MeOD, 400 MHz, J/Hz). d (ppm) 7.59 (1H, dd, J=8.5, 2.1 Hz, H-6'), 7.05 (1H, d, J=8.5 Hz, H-5'), 6.41(1H, d, J=1.8 Hz, H-6), 6.18 (1H,d, J=1.8 Hz, H-8), 3.91 (3H, s, 7- OCH3), 3.76 (3H, s, 4'-OCH3) [23].

(6) **Quercetin**, $C_{15}H_{10}O_7$, yellow powder, mp: 313～314 °C, ^{1}H NMR (400 MHz, MeOD, d, ppm, J/Hz). d: 7.72 (1H, d, J=2.1 Hz, H-2'), 7.65(1H, dd, J=8.5, 2.1 Hz, H-6'), 6.87 (1H, d, J=8.3 Hz, H-5'), 6.38 (1H, d, J=2.0 Hz, H-8), 6.18 (1H, d, J=2.0 Hz, H-6) [24].

DPPH and ABTS free radical scavenging effect

The isolated flavonoids or flavonoid glycosides were evaluated for their antioxidant effect using DPPH and ABTS free radical scavenging methods

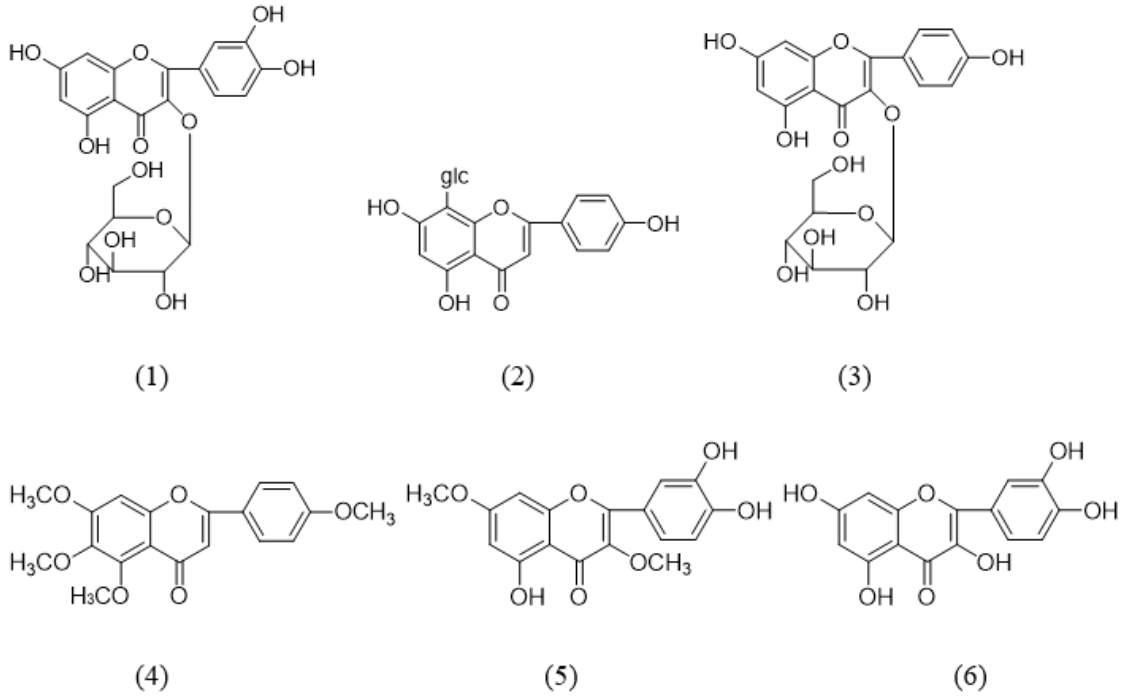

Figure 1: Chemical structure of flavonoids or flavonoid glycosides isolated from BP. (1) Isoquercitrin (2) Vitexin, (3) Astragalin, (4) 5,6,7,4'-tetramethoxylflavone, (5) 5,3',4'-trihydroxy-3,7-dimethoxylflavone, (6) Quercetin

Table 1: IC_{50} values (μmol/L) in the antioxidant assays of DPPH scavenging and ABTS scavenging of isolated flavonoids or flavonoid glycosides

Compound	Molecular weight	DPPH Radical Scavenging Activity	ABTS Radical Scavenging Activity
1	464	15.2±0.4	21.3±1.7
2	432	ND	ND
3	448	199.8±2.3	212.3±1.6
4	342	ND	ND
5	330	43.1±0.3	77.6±0.4
6	302	10.7±0.2	17.5±2.6
BHT	220	58.6±1.4	79.4±2.6

ND = not determined (maximum level of inhibition below 50 %)

DPPH-radical scavenging activity is a rapid means used to compare the antioxidant capacity of different natural compounds. When treated with the isolated compounds, DPPH solution, initially deep violet in color, turned pale yellow in the presence of non-radical form of DPPH-H [25]. As seen in Table 1, compounds 1, 5 and 6 showed significant DPPH scavenging activity, with IC_{50} values of 15.2 μmol/L, 43.1 μmol/L and 10.7 μmol/L, respectively. All of them were found to be more efficient than the positive control BHT, with IC_{50} value of 58.6 μmol/L. However, compound 3 exerted moderate DPPH scavenging activity, with IC_{50} value of 199.8 μmol/L. Furthermore, compounds 2 and 4 were documented to present the lowest scavenging activity. Their radical scavenging ability decreased in the following decreasing order: 6 > 1 > 5 > BHT > 3 > 2 and 4.

ABTS is converted to its radical cation ($ABTS^{\bullet+}$) by reacting with a strong oxidizing agent (e.g., potassium permanganate, potassium persulfate). This radical cation is blue in color and absorbs light at 734 nm, which will be converted back to its colorless neutral form by hydrogen-donating antioxidant [26]. The inhibitive efficiency of $ABTS^{\bullet+}$ by the assayed compounds is presented in Table 1. Our data showed that quercetin (compound 6) was the most effective in ABTS free radical-scavenging assay with IC_{50} value of 17.5 μmol/L. Nevertheless, ABTS radical-scavenging activity of compound 3 was lower than BHT. Moreover, compounds 2 and 4 were also found to show the weakest scavenging activity. The radical scavenging ability decreased in the order: 6 > 1> 5≈BHT>3 > 2 and 4.

Anti-proliferative activity against RKO cells

MTT assay was conducted to evaluate the growth inhibitory effect of the compounds on the cell viability of human colon cancer cell line RKO. As provided in Fig. 2 A, various concentrations (50, 100, 200 and 400 μmol/L) of compounds 1, 2, 3 and 6 exhibited a significant cytotoxic effect against RKO cells in a dose-dependent manner for 24 h ($p < 0.05$). The IC_{50} values of the

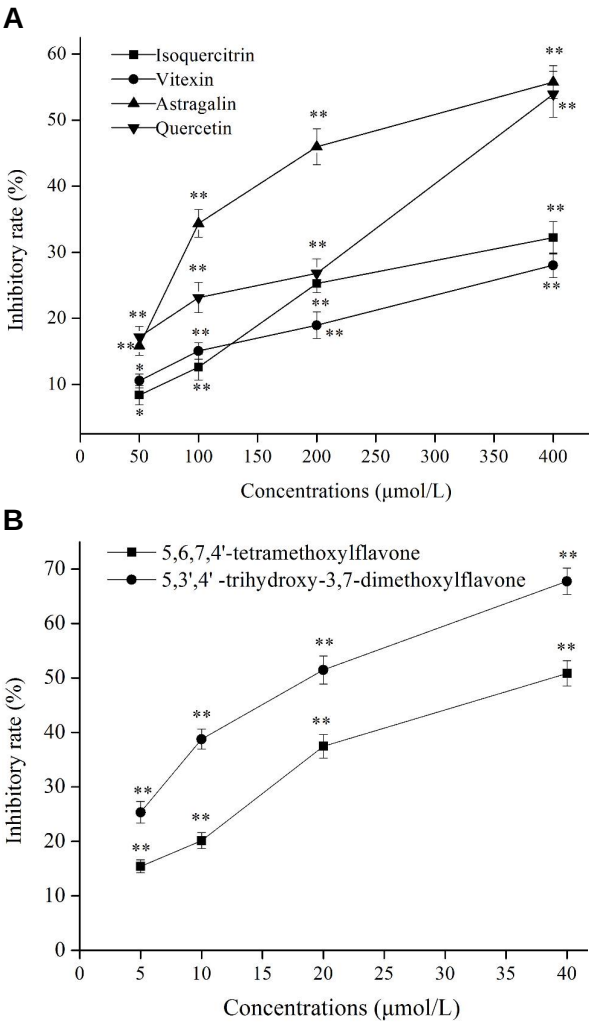

Figure 2: Inhibitory effect of the isolated compounds against RKO cells by MTT assay for 24 h. A: various concentrations (50, 100, 200 and 400 μmol/L) of isoquercitrin, vitexin, astragalin, and quercetin; B: various concentrations (5, 10, 20 and 40 μmol/L) of 5,6,7,4'-tetramethoxylflavone and 5, 3', 4'-trihydroxy-3, 7-dimethoxylflavone

compounds 3 and 6 were 265.34 µmol/L and 452.48 µmol/L, respectively. However, lower cytotoxicity was observed in compounds 1 and 2 (both their IC_{50} values were > 500 µmol/L). Compared with the four aforementioned compounds, compound 4 and 5 were shown to display more potent anti-proliferative activity, with IC_{50} values of 39.08 and 17.68 µmol/L, respectively ($p < 0.01$), as shown in Fig. 2 B. Taken together, our result suggested that the flavonoid compounds contained in BP were partially responsible for its biological/ pharmacological activities.

DISCUSSION

BP, as a rich source of flavonoids, is used to treat periappendicular abscess in traditional Chinese medicine, which is also employed as a folk medicine and a major ingredient of herbal tea in Taiwan believed to be able to prevent inflammation and cancer [15]. Recently, our consecutive phytochemical investigation led to 6 flavonoid compounds from this plant, including isoquercitrin (1), vitexin (2), astragalin (3), 5,6,7,4'-tetramethoxylflavone (4), 5,3',4'-trihydroxy-3,7-dimethoxylflavone (5) and quercetin (6), whose chemical structures are presented in Figure 1.

The antioxidant capacities of these isolated compounds had been evaluated by using DPPH and ABTS free radical scavenging assays. The result from antioxidant evaluation indicated that quercetin was the most effective in both DPPH (IC_{50} value of 10.7 µmol/L) and ABTS (IC_{50} value of 17.5 µmol/L) free radical-scavenging assays. However, vitexin (2) and 5,6,7,4'-tetramethoxylflavone (4) displayed low radical-scavenging activity, with maximum level of inhibition below 50 %, as seen in Table 1. It was interesting to reveal the structure-activity relationship. Flavonoids exhibit their antioxidative functions through two major mechanisms viz. hydrogen atom transferring and electron donation [27]. The antioxidant activity of flavonoids is due to their molecular structure. The number and position of hydroxyl groups on the B and A rings are the key factors for the antioxidant activity. As to the C-glycoside, with glucose at C-6 or C-8 the antioxidant activity decreases due to steric hindrance by the glucose moiety [28]. From this point of view, the absence of hydroxyl groups in 5, 6, 7, 4'-tetramethoxylflavone (4) and the glucose at C-8 of vitexin (2) were responsible for the low radical scavenging activity, respectively. Moreover, the presence of an ortho-dihydroxy (3`, 4`-di OH) moiety on the B ring improve the radicals scavenging activity and the antioxidant potential of the flavonoids due to hydrogen bonding and electron delocalization which have influence on stabilization of the flavonoid phenoxyl radicals [29]. Therefore, the high contribution of an ortho-dihydroxy moiety on the B ring of isoquercitrin (1), 5,3',4'-trihydroxy-3,7-dimethoxylflavone (5) and quercetin (6) led to their significant antioxidant activity. Additionally, it was reported that the presence of a saccharide group at C-3 in the C ring had little influence on antioxidant activity [30]. Hence, astragalin (2) demonstrated relatively lower effect mainly due to the lack of an ortho-dihydroxy moiety.

The anti-proliferative activity of the isolated flavonoids was evaluated against RKO cells by using MTT method. Various concentrations (50, 100, 200 and 400 µmol/L) of isoquercitrin (1), vitexin (2), astragalin (3), and quercetin (6) exhibited a significant cytotoxic effect against RKO cells in a dose-dependent manner for 24 h. But all of them were much less effective than 5, 6, 7, 4'-tetramethoxylflavone (4) and 5, 3', 4'-trihydroxy-3, 7-dimethoxylflavone (5), with IC_{50} value of 39.08 µmol/L and 17.68 µmol/L, respectively ($p < 0.01$). There is increasing evidence that methoxylated flavones have chemopreventive properties superior to the common unmethylated flavonoids or polyphenols. For example, 5, 7, 4′-trimethoxyflavone was about eight times more potent than apigenin [31], which is in agreement with our previous findings that 3, 3′-dimethylquercetin exhibited much more cytotoxicity than quercetin [15]. Thus, 5, 6, 7, 4'-tetramethoxylflavone and 5, 3', 4'-trihydroxy-3,7-dimethoxylflavone displayed more remarkable growth inhibitory effect than the other four flavonoids in our study.

CONCLUSION

BP is a noteworthy material containing a series of bioactive flavonoid compounds, thus suggesting that it can be utilized as a potential health supplement and an easily available source of natural antioxidants, as well as an effective material in pharmaceutical applications.

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REFERENCES

1. *Awah FM, Uzoegwu PN, Oyugi JO, Rutherford J, Ifeonu P, Yao XJ, Fowke KR, Eze MO. Free radical scavenging activity and immunomodulatory effect of Stachytarpheta angustifolia leaf extract. Food Chem 2010; 119: 1409–1416*
2. *Hursting SD, Slaga TJ, Fischer SM, DiGiovanni J, Phang JM. Mechanism-based cancer prevention approaches: targets, examples, and the use of transgenic mice. J Natl Cancer Inst 1999; 91: 215–225.*
3. *Mattern J, Volm M. Imbalance of cell proliferation and apoptosis during progression of lung carcinomas. Anticancer Res 2004; 24: 4243-4246.*
4. *Wu JG, Peng W, Yi J, Wu YB, Chen TQ, Wong KH, Wu JZ. Chemical composition, antimicrobial activity against Staphylococcus aureus and apro-apoptotic effect in SGC-7901 of the essential oil from Toona sinensis (A. Juss.) Roem. leaves. J Ethnopharmacol 2014; 154: 198-205.*
5. *Kinghorn AD, Chin YW, Swanson SM. Discovery of natural product anticancer agents from biodiverse organisms. Curr Opin Drug Discov Devel 2009; 12: 189-196.*
6. *Wang J, Lou J, Luo C, Zhou L, Wang M, Wang L. Phenolic compounds from Halimodendron halodendron (Pall.) Voss and their antimicrobial and antioxidant activities. Int J Mol Sci 2012, 13(9): 11349-11364.*
7. *Theodoratou E, Kyle J, Cetnarskyj R, Farrington SM, Tenesa A, Barnetson R, Porteous M, Dunlop M, Campbell H. Dietary flavonoids and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2007; 16: 684-693.*
8. *Amado NG, Predes D, Fonseca BF, Cerqueira DM, Reis AH, Dudenhoeffer AC, Borges HL, Mendes FA, Abreu JG. Isoquercitrin suppresses colon cancer cell growth in vitro by targeting the Wnt/β-catenin signaling pathway. J Biol Chem 2014; 289: 35456-35467.*
9. *Ranelletti FO, Maggiano N, Serra FG, Ricci R, Larocca LM, Lanza P, Scambia G, Fattorossi A, Capelli A, Piantelli M. Quercetin inhibits p21-RAS expression in human colon cancer cell lines and in primary colorectal tumors. Int J Cancer 2000; 85: 438-445.*
10. *Haratifar S, Meckling KA, Corredig M. Antiproliferative activity of tea catechins associated with casein micelles, using HT29 colon cancer cells. J Dairy Sci 2014; 97: 672-678.*
11. *Alvarez A, Pomar F, Sevilla, Montero MJ. Gastric antisecretory and antiulcer activities of an ethanolic extract of Bidens pilosa L. var. radiata Schult. Bip. J Ethnopharmacol 1999; 67: 333-340.*
12. *Chien SC, Young PH, Hsu YJ, Chen CH, Tien YJ, Shiu SY, Li TH, Yang CW, Marimuthu P, Tsai LF, Yang WC. Anti-diabetic properties of three common Bidens pilosa variants in Taiwan. Phytochem 2009; 70: 1246-1254.*
13. *Yuan LP, Chen FH, Ling L, Dou PF, Bo H, Zhong MM, Xia LJ. Protective effects of total flavonoids of Bidens pilosa L. (TFB) on animal liver injury and liver fibrosis. J Ethnopharmacol 2008; 116: 539-546.*
14. *Wu JG, Wan ZX, Wu YB, Peng W, Zheng CJ, Wu JZ. Chemical constituents of Bidens pilosa var. radiate. Chem Nat Compd 2013; 49: 759-760.*
15. *Wu J, Wan Z, Yi J, Wu Y, Peng W, Wu J. Investigation of the extracts from Bidens pilosa Linn. var. radiata Sch. Bip. for antioxidant activities and cytotoxicity against human tumor cells. J Nat Med 2013; 67: 17-26.*
16. *Sarah K Nanyonga, Andy R Opoku, Francis B Lewu, Opeoluwa O Oyedeji, Moganavelli Singh, Adebola O Oyedeji. Antioxidant activity and cytotoxicity of the leaf and bark extracts of Tarchonanthus camphorates. Trop J Pharm Res 2013; 12: 377-383.*
17. *Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999; 26: 1231–1237.*
18. *Wu JG, Ma L, Zhang SY, Zhu ZZ, Zhang H, Qin LP, Wei YJ.. Essential oil from rhizomes of Ligusticum chuanxiong induces apoptosis in hypertrophic scar fibroblasts. Pharm Biol 2011; 49: 86-93.*
19. *Peng W, Han T, Liu QC, Qin LP. Chemical constituents of the flower of Fritillaria thunbergii. Chem Nat Compd 2012; 48: 491-492.*
20. *Li HM, Hwang SH, Kang BG, Hong JS, Lim SS. Inhibitory effects of Colocasia esculenta (L.) Schott constituents on aldose reductase. Molecul 2014; 19: 13212-13224.*
21. *Lu YR, Foo LY. The polyphenol constituents of grape pomace. Food Chem 1999; 65:1-8.*
22. *Wang J, Liu X, Dong BP, Lv X, Xu WC. Synthesis and characterization of scutellarein. Chem Res Appl 2012; 24: 480-484.*
23. *Duan XF, Zhang ZB, Duan XH. A modified synthesis of 5,3',4'-Trihydroxyl-6,7-dimethoxyflavone. Chin J Orga Chem 2003; 23: 353-355.*
24. *Liu H, Mou Y, Zhao J, Wang J, Zhou L, Wang M, Wang D, Han J, Yu Z, Yang F. Flavonoids from Halostachys caspica and their antimicrobial and antioxidant activities. Molecules 2010; 15: 7933-7945.*
25. *Yang H, Gu Q, Gao T, Wang X, Chue P, Wu Q, Jia X. Flavonols and derivatives of gallic acid from young leaves of Toona sinensis (A. Juss.) Roemer and evaluation of their antioxidant capacity by chemical methods. Pharmacogn Mag 2014; 10: 185-190.*
26. *Boligon AA, Machado MM, Athayde ML. Technical evaluation of antioxidant activity. Med chem 2014; 4: 517-522.*
27. *Ezzati Nazhad Dolatabadi J, Mokhtarzadeh A, Ghareghoran SM, Dehghan G. Synthesis, characterization and antioxidant property of quercetin-Tb(III) complex. Adv Pharm Bull 2014; 4: 101-104.*
28. *Zeng P, Zhang Y, Pan C, Jia Q, Guo FJ, Li YM, Zhu WL, Chen KX. Advances in studying of the pharmacological activities and structure–activity relationships of natural*

C-glycosylflavonoids. Acta Pharm Sin B 2013; 3: 154–162.

29. *Anantachoke N, Kitphati W, Mangmool S, Bunyapraphatsara N. Polyphenolic Compounds and antioxidant activities of the leaves of Glochidion hypoleucum. Nat Prod Commun 2015; 10: 479-482.*
30. *Wu YB, Zheng LJ, Wu JG, Chen TQ, Yi J, Wu JZ. Antioxidant activities of extract and fractions from Receptaculum Nelumbinis and related flavonol glycosides. Int J Mol Sci 2012; 13: 7163-7173.*
31. *Walle T. Methoxylated flavones, as superior cancer chemopreventive flavonoid subclass? Semin Cancer Biol 2007; 17: 354-362.*