Antioxidant and Anticancer Activities of Raspberry Extracts

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Abstract: The raspberry (*Rubus idaeus*) is an economical important berry crop that contains phytochemicals such as polyphenols and flavonoids with potential health benefits. This study addresses the antioxidant and anticancer effects of raspberry and its root extracts. Raspberry and raspberry root were extracted with ethanol, and separated into petroleum ether, chloroform, ethyl acetate, n-butyl alcohol and water fraction. Most extracts showed the powerful activities to scavenge DPPH radical, eliminate hydroxyl free radical ion, and inhibit the growth of human cancer cells, suggesting their promising application on health care.

Keywords: Raspberry, antioxidant, anticancer.

1. INTRODUCTION

Berry fruits are renowned for their high concentration of phytonutrients such as vitamins, anthocyanins, flavonols, and hydroxybenzoic acids with elucidated antioxidant, anti-inflammatory, anti-proliferative, antimicrobial, vasodilatory, anti-aging and anti-heart disease activities, and are thus considered as a function food ingredient [1-6]. Raspberry (*Rubus ideaus*) is known for naturally rich sources of dietary antioxidants, such as anthocyanins, flavonoids (quercetin, flavan-3-ols and its oligomers), phenolic acids (ellagic acid and ellagitannins), lignans and tannins [2, 7, 8]. In addition, raspberries also contain other healthy nutrients, such as carbohydrates (sugars and dietary fiber), vitamins especially vitamin C, E, K, B_5 and choline, and abundant trace metals (calcium, magnesium, phosphorus, potassium, zinc, iron and manganese) [2, 7-10]. The phytochemicals in plant tissues accountable for the antioxidant capacity can largely be attributed to flavonoids, phenolic acids and anthocyanins [1, 4, 11, 12]. Besides for antioxidant properties, raspberries have been reported other beneficial bioactivities, including antimicrobial, antiviral, anti-inflammatory, anti-neurodegenerative, anti-proliferative and anticancer activities [11, 13-17]. Not only fruit but also roots and leaves of raspberry have been used as traditional medicine to treat sore throats, wound cleaning, morning sickness, colic pain and childbirth-related muscle spasms and so on [18-21]. In this study, we investigated the antioxidant and anticancer activities of different solvent extracts of raspberries and its roots.

2. MATERIALS AND METHODS

2.1. Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) and N-acetly-L-cysteine (NAC) were purchased from Sigma-Aldrich. (St. Louis, MO). Hydrogen peroxide 30% solution, iron (Ⅱ) sulfate and 1,10-phenanthroline were purchased from BBI Life Sciences Co., Ltd. (Shanghai, China).

2.2. Cell and Cell Culture

HCT 116 (human colon adenocarcinoma) and H1299 (human lung adenocarcinoma) cell lines obtained from ATCC were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100U/mL penicillin, 100μg/mL streptomycin. All cell cultures were maintained at 37 ˚C in a humidified atmosphere of 5% CO_2.

2.3. DPPH Radical Scavenging Assay

The DPPH radical-scavenging activities of all extracts was determined as previously described [22-24]. Briefly, 20 μL of extracts solution was mixed with 180 μL of DPPH solution at various concentrations into the 96-well plate. The plate was incubated at 37 ˚C for 30 min in dark, and absorbance at 517 nm was measured by plate reader.

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2.4. Hydroxyl Free Radical Scavenging Assay

The hydroxyl radical scavenging properties of all extracts was carried out by measuring the hydroxyl radicals generated from the 1,10-phenanthroline/ Fe^{2+}/H_2O_2 system based on Fenton reaction [25-27]. The reaction mixture contained the following reagents: 20 µL of the extracts and NAC at various concentration, 15 µL of 5 mM 1,10-phenanthroline, 40 µL of PBS, 10 µL of 7.5 mM $FeSO_4$, with or without 10 µL 1% H_2O_2, and distilled water to final volume of 200 µL. The reaction mixture was incubated at 37 ˚C for 30 min, and absorbance at 510 nm was measured by plate reader.

2.5. Cell Viability Assay

Cells were seeded into a 96-well plate at a density of 5 × 10^3 cells/well and treated with various concentrations of extracts for 72 hours. MTT was added to each well at a final concentration of 0.5 mg/ml. After incubation for 4 hours, formazan crystals were dissolved in 100 µl of DMSO, and absorbance at 570 nm was measured by plate reader. The concentrations required to inhibit growth by 50% (IC_{50}) were calculated from survival curves as previously described [28].

2.6. Statistical Analysis

All experiments were carried out at least 3 times and results are shown as the mean ± SD.

3. RESULTS

The DPPH radical scavenging method is one of the most widely used assay to test the antioxidant activity [22]. To obtain the optimal reaction time, we firstly tested the DPPH radical scavenging activity of NAC which is a common antioxidant agent widely used as standards [29, 30]. As shown in Figure **1**, the free radical-scavenging activity of NAC increased with time up to 50 mins, and the IC_{50} for NAC to scavenge DPPH radical was 89.23 µM at the time of 50 mins. The DPPH radical-scavenging activities of all the extracts are shown in Table **1**. The results showed that at a lower concentration (0.1 mg/ml), ethanol, ethyl acetate and water extracts fractions of raspberry and its roots, n-butyl alcohol fraction of raspberry and chloroform fractions of raspberry root showed no differences in the DPPH scavenging activity in comparison with NAC, and petroleum ether extracts, chloroform extracts of raspberry and n-butyl alcohol possessed a significantly lower DPPH radical scavenging ability. However, at a higher concentration (1 mg/ml), the DPPH radical scavenging characteristics of the extracts and NAC were similar, except for water fractions and petroleum ether fraction of raspberry root. These results indicate that the extracts of raspberry have a noticeable effect on scavenging DPPH radicals and may be related to the high phenolic and flavonoids constituents present.

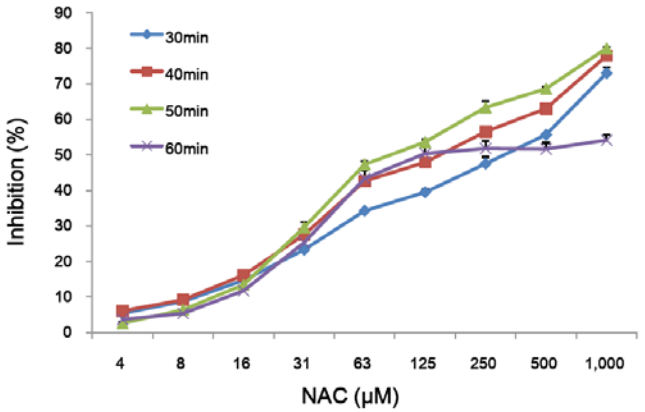


Figure 1: The dose- and time-effects of NAC on anti-DPPH oxidation activity. Activity was measured by the scavenging of DPPH radicals and expressed as percent inhibition. Date were mean ± S. D. of three independent experiments.

Hydroxyl radicals are highly reactive and consequently short half-life of approximately 10^{-9} seconds. Hydroxyl radicals play an important role in radical chemistry as well as oxidative stress. Thus, the highly reactive hydroxyl radicals can cause oxidative damage to DNA, proteins and lipids. The hydroxyl radical-scavenging capacity of the extracts was measured using the modified method from Kunchandy and Richmond [25-27]. As shown in Table **2**, all extracts are good scavenger of hydroxyl radical at both low and high concentration in comparison with NAC, except for the petroleum ether fractions. Comparing with the extracts of raspberry with the extracts of raspberry roots, the raspberry extracts exhibited higher hydroxyl radical scavenging activity in general.

To investigate the effects of all extracts on cell proliferation, two cancer cell line H1299 and HCT116 were tested with MTT assay. As shown in Table **3** and Figure **2**, most extracts could inhibit the proliferation of both cell lines in a dose-dependent manner.

4. DISCUSSION

Raspberries are good sources of nature antioxidant [2, 7, 8]. Extracts of berries of several cultivars of blackberries, rasperries and strawberried showed a significantly high scavenging activity toward chemically

Table 1: DPPH Scavenging Power of the Ethanol Extracts and Different Polarity Fractions of Raspberry and of NAC as Standard in Dose- and Time-Effect

Reaction time (minutes)	30		40		50	
Extracts (mg/ml)	0.1	1	0.1	1	0.1	1
Ethanol extract of Ras.	46.38±3.13	69.17±4.92	48.10±3.57	72.19±2.73	49.57±2.67	72.54±3.10
Petroleum ether of Ras.	25.50±5.0	79.02±0.30	27.44±5.66	79.32±0.29	29.23±5.90	79.42±0.25
Chloroform of Ras.	36.60±2.00	78.56±0.68	37.75±0.81	79.21±0.17	37.57±0.82	79.39±0.09
Ethyl acetale of Ras.	47.45±2.60	81.37±0.37	47.51±2.26	81.28±0.31	47.39±2.39	81.26±0.32
n-butyl alcohol of Ras.	49.74±4.03	79.42±0.38	50.53±4.15	79.64±0.19	51.19±4.15	79.57±0.22
Water of Ras.	44.40±0.84	56.30±2.91	48.98±2.17	58.72±2.08	51.16±0.29	59.02±1.93
Ethanol extract of Ras. Root.	41.67±4.23	81.26±1.57	42.31±4.37	81.48±1.72	42.30±4.15	81.52±1.77
Petroleum ether of Ras.Root.	1.88±2.68*	41.27±0.35*	3.91±2.47*	43.00±0.36*	6.23±2.64*	44.42±0.63*
Chloroform of Ras. Root.	32.40±8.22	77.38±0.29	34.46±8.00	78.28±0.23	35.24±7.33	78.40±0.28
Ethyl acetale of Ras. Root.	42.58±1.13	83.74±0.22	42.08±1.04	83.97±0.06	42.08±1.20	84.04±0.02
n-butyl alcohol of Ras. Root.	35.50±0.85	80.94±0.20	36.23±0.70	81.20±0.03	37.73±0.49	81.21±0.01
Water of Ras. Root.	36.30±1.66	69.88±0.72	39.15±2.12	70.03±0.40	40.10±1.39	72.23±0.52
NAC	37.4±1.0	73.02±1.66	45.79±1.21	77.89±1.51	51.08±0.81	80±0.29

Each value is expressed as mean ±SD. *P<0.05, *vs* NAC.

Table 2: Hydroxyl Radical-Scavenging Power of the Ethanol Extracts and Different Polarity Fractions of Raspberry and of NAC as Standard

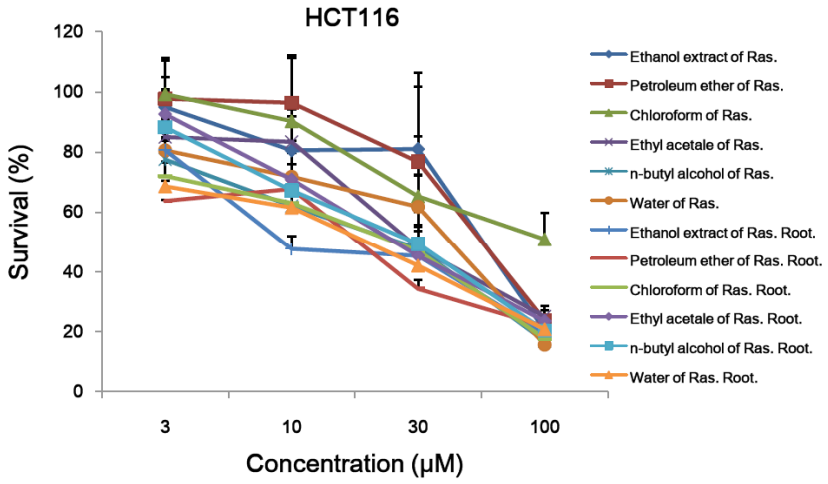
Extracts (mg/ml)	0.1	1
Ethanol extract of Ras.	66.35±1.45*	246.88±29.16*
Petroleum ether of Ras.	-58.81±1.21*	-7.02±0.25*
Chloroform of Ras.	46.81±1.28*	143.55±0.46*
Ethyl acetale of Ras.	123.62±5.68*	427.43±15.96*
n-butyl alcohol of Ras.	153.34±3.23*	434.11±3.00*
Water of Ras.	11.61±0.06*	294.23±16.75*
Ethanol extract of Ras. Root.	91.24±3.64*	85.90±4.48*
Petroleum ether of Ras. Root.	19.23±0.66	-76.22±0.10*
Chloroform of Ras. Root.	38.03±0.64*	103.11±3.82*
Ethyl acetale of Ras. Root.	115.23±7.63*	166.66±1.95*
n-butyl alcohol of Ras. Root.	86.00±6.64*	364.75±3.43*
Water of Ras. Root.	182.41±13.97*	211.77±0.47*
NAC	22.51±0.22	55.82±2.67

Each value is expressed as mean ±SD. *P<0.05, *vs* NAC.

Table 3: Summary of IC_{50} Values of Extracts against H1299 and HCT116 Cancer Cells

Extracts (μg/ml)	HCT116	H1299
Ethanol extract of Ras.	68.04 ±1.32	65.89 ±14.00
Petroleum ether of Ras.	27.62 ±12.01	65.39 ±23.73
Chloroform of Ras.	20.45 ±2.00	>100
Ethyl acetale of Ras.	25.63 ±17.83	28.90 ±7.89
n-butyl alcohol of Ras.	48.47 ±0.1	26.97 ±16.83
Water of Ras.	72.50 ±11.76	47.95 ±12.47
Ethanol extract of Ras. Root.	67.97±2.97	9.51 ±3.18
Petroleum ether of Ras. Root.	<3	20.61 ±0.07
Chloroform of Ras. Root.	67.16 ±7.62	26.32 ±0.2
Ethyl acetale of Ras. Root.	51.47 ±4.01	26.38 ±14.82
n-butyl alcohol of Ras. Root.	13.27 ±2.72	29.36 ±5.35
Water of Ras. Root.	51.10 ±22.95	21.86 ±19.40

Each value is expressed as mean±S.D.



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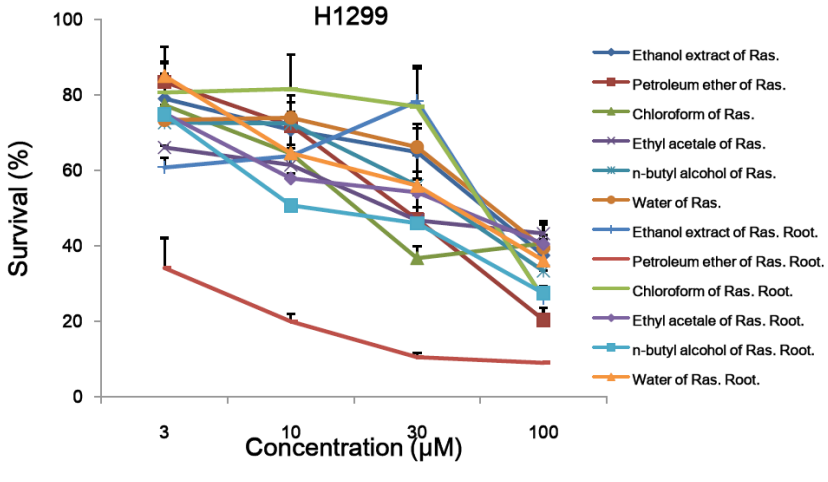

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Figure 2: Effect of the extracts on the growth of H1299 and HCT116 cancer cells *in vitro*. Cells were treated with various concentrations of extracts for 72 h and cell survival was determined by MTT assay. Date were mean ± S. D. of three independent experiments.

generated superoxide radicals [5, 15]. High levels of antioxidants have shown to multiple benefits to human health [6, 13, 18, 31]. During the last decade, berries attracted increasing attention due to anti-proliferative activities in human cancer cells, such as human liver cancer, cervical cancer, laryngeal cancer and colonrectal cancer [10, 13, 16-20, 31]. In this study, we demonstrated the antioxidant and anti-proliferative activity of raspberry extracts. The different fractions and root of raspberry contained a considerable amount of antioxidants and exhibited some DPPH radical-scavenging activity and hydroxyl free radical elimination activity. Furthermore, most extracts showed anti-proliferative capacity in cancer cells. The phytochemicals in the extracts accountable for the antioxidant and anti-proliferative activities may be flavonoids and phenolic. In addition, based on their antioxidant and anticancer activities, the raspberry extracts should be considered as a potential food ingredient as well as novel therapeutic agent. Therefore, further works are necessary to estimate the chemical compositions and health benefits of these extracts obtained from raspberry.

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