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Substituting one *Paris* for another? *In vitro* cytotoxic and *in vivo* antitumor activities of *Paris forrestii*, a substitute of *Paris polyphylla* var. *yunnanensis*

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Ethnopharmacological relevance:

Chong-lou (*Paris polyphylla* var. *yunnanensis* or *P. polyphylla* var. *chinensis*) is traditionally used as an anticancer medicine in China. It is also the material basis of some Chinese patent anticancer medicines, such as Gan-Fu-Le capsules, Bo-Er-Ning capsules, Lou-Lian capsules, Ruan-Jian oral liquid, and Qi-Zhen capsules. *P. forrestii*, a substitute for Chong-lou, is planted at a large scale in the Yunnan Province of China.

Aim of the study:

To clarify the active chemical constituents of *P. forrestii* and evaluate the *in vitro* and *in vivo* anticancer activities of the total saponins from *P. forrestii*.

Materials and methods:

The total saponins of *P. forrestii* were extracted and separated to yield pure compounds by chromatographic techniques, and the structures of the isolates were elucidated by spectroscopic methods. The cytotoxicity of the crude extracts, total saponins, and chemical constituents were evaluated using an MTS assay. *In vivo* antitumor activities of the total saponins from *P. forrestii* were measured using H22 tumor-bearing mice by intraperitoneal (ip) administration.

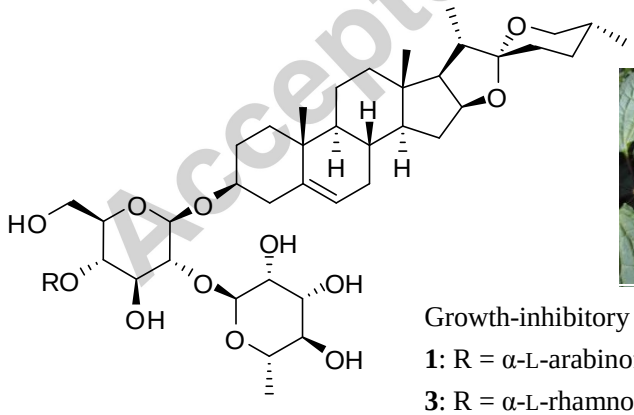
Results:

Eight compounds, including polyphyllin D (**1**), formosanin C (**2**), dioscin (**3**), diosgenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**4**), paris saponin H (**5**), pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (**6**), pariposide A (**7**), and crustecdysone (**8**), were isolated from the total saponins of *P. forrestii*. The total saponins and compounds **1**–**6** showed significant inhibitory activity against the growth of the HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines. The total saponins from *P. forrestii* had a tumor-inhibitory effect in H22 tumor-bearing mice upon ip (2.25 mg/kg dose) administration, with an inhibition rate of 42.6% compared with cisplatin (ip, 2 mg/kg dose, 53.9% inhibition rate).

Conclusion:

The results support that *P. forrestii* could be a substitute for *P. polyphylla* var. *yunnanensis* as an anticancer medicine.

Graphical Abstract



Growth-inhibitory activity against SMMC-7721 cells
1: R = α-L-arabinofuranosyl; IC_{50}=0.61 μM
3: R = α-L-rhamnopyranosyl; IC_{50}=0.79 μM

Keywords:

Melanthiaceae; *Paris forrestii*; saponins; cytotoxicity; anticancer

1. Introduction

Cancer remains the second leading cause of death worldwide. It is unsatisfactory that over the past few decades, treatment for cancer has not progressed as fast as expected, with only 50% of newly diagnosed patients cured (Arends, 2010). Drug resistance is a common cause of treatment failure in cancer (Kumar et al., 2013). Therefore, development of new anticancer drugs is of importance in clinical therapy, with natural products considered to be important sources for the development of new anticancer drugs (Newman and Cragg, 2016).

The genus *Paris* L. (Melanthiaceae) contains 32 species and more than 10 varieties (Ji et al., 2017; Li et al., 2017a; Li et al., 2015; Liu et al., 2017; Wang et al., 2017b; Yang et al., 2017). *P. polyphylla* var. *yunnanensis* (Franch.) Hand.-Mazz. and *P. polyphylla* var. *chinensis* (Franch.) H. Hara, also known as Chong-lou in China, are famous traditional Chinese medicines included in the Chinese Pharmacopoeia (Wang et al., 2015b). *P. polyphylla* var. *yunnanensis* is traditionally used as an anticancer medicine in China (Su and Wei, 1983). *P. polyphylla* var. *chinensis* is also a traditional Chinese medicine used to treat brain tumors, lung cancer, leukemia, osteosarcoma, myosarcoma, malignant lymphoma, and benign tumors (Shanghai Office for Cancer Prevention and Control, 1977). In the Sichuan Province of China, the Yi people use *P. polyphylla* var. *stenophylla* Franch. to treat gynaecological cancer (Yin and Zhang, 2010). *P. polyphylla* Smith is also used as an anticancer medicine in Nepal (Shrestha et al., 2016). In China, Chou-lou is a source of some Chinese patent anticancer medicines, such as Gan-Fu-Le capsules (Qiu et al., 2016; Wu et al., 2014),

Bo-Er-Ning capsules (Zhu et al., 2016), Lou-Lian capsules (Chen et al., 1999), Ruan-Jian oral liquid (Li, 2008), and Qi-Zhen capsules (Tang, 2016). Additionally, *P. polyphylla* var. *yunnanensis* and *P. polyphylla* var. *chinensis* are also used to treat bleeding, snake bites, and other conditions in traditional Chinese folk medicine (Wang et al., 2015b). According to previous reports, steroidal saponins are the major active chemical constituents of *Paris* plants (Wang et al., 2015b).

Because of a shortage of wild populations, *Paris* plants are now cultivated at a large scale in China. For example, in Nujiang in the Yunnan Province, there was approximately 200 hm^2 of planting area for *Paris* plants in the year 2014 (Wang et al., 2015b). The plants cultivated were mainly *P. polyphylla* var. *yunnanensis* and *P. forrestii* (Takht.) H. Li. The latter was thought to be a medicinal substitute for the former. In addition to our existing patents (Wang et al., 2016a; Wang et al., 2015a; Wang et al., 2015c; Wang et al., 2016b) and a recent publication documenting antitumor activity (Chen et al, 2017), there are few reports on the analgesic, anti-inflammatory, and hemostatic effects of extracts from *P. forrestii* (Li et al., 2017b) and saponins in the plant detected by high-performance liquid chromatography (HPLC) (Huang et al., 2017; Yuan et al., 2017). In this paper, the *in vitro* cytotoxic activity against five cancer cell lines of the crude extracts, total saponins, and pure compounds from the rhizomes of *P. forrestii* and the *in vivo* antitumor activity against the mouse hepatocellular carcinoma H22 cell line by the total saponins from *P. forrestii* are reported.

2. Experimental

2.1. General

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were collected on Bruker AM-400, DRX-500 and Avance III-600 spectrometers (Bruker Bio-Spin GmbH, Rheinstetten, Germany) with tetramethylsilane (TMS) as an internal standard. Electrospray ionization mass spectrometry (ESIMS) analyses were performed on an API QSTAR Pulsar 1 spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA). Silica gel G (80–100 and 300–400 mesh, Qingdao Meigao Chemical Co., Ltd., Qingdao, China), C_{18} silica gel (40–75 μm, Fuji Silysia Chemical Ltd., Aichi, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography, and silica gel GF_{254} (Qingdao Meigao Chemical Co., Ltd.) was used on pre-coated plates for preparative thin layer chromatography (TLC). TLC spots were visualized under ultraviolet (UV) light at 254 nm and by dipping the plates into 5% H_2SO_4 in alcohol followed by heating. Semi-preparative HPLC was performed on an Agilent 1200 series system (Agilent Technologies, Santa Clara, USA) equipped with a diode array detector and an Agilent Zorbax SB-C_{18} column (5.0 μm, ϕ 9.4×250 mm).

2.2. Plant material

The rhizomes of *P. forrestii* were collected from Nujiang in the Yunnan Province, People's Republic of China in September 2014. Based on the taxonomic system in the publication *Flora of China*, the plant was identified by one of the authors (Prof. Heng Li), and a voucher specimen (No. yj-023-A-2) was deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy

of Sciences.

2.3. Extraction and isolation

The air-dried, powdered *P. forrestii* plant (4.4 kg) was exhaustively extracted with 95% EtOH (4×13 L) at room temperature. The EtOH extract (550 g) was suspended in H_2O (2 L) and further partitioned with EtOAc (3×2 L) and *n*-butanol (3×2 L) to obtain the EtOAc-soluble fraction (A, 19 g) and the *n*-butanol-soluble fraction (B, 290 g), respectively.

One hundred and eighty grams of fraction B was subjected to column chromatography (silica gel; $CHCl_3$/MeOH, 10:1→1:1, v/v) to yield fractions B1 (14 g), B2 (101 g), B3 (45 g), and B4 (1.0 g). Fraction B2 was separated on an RP-18 silica gel column and eluted with MeOH/H_2O (10%→100%) to yield sub-fractions B2-1 (2.0 g), B2-2 (0.5 g), B2-3 (0.8 g), B2-4 (4.6 g), B2-5 (16.1 g), and B2-6 (1.0 g).

One hundred and fifty milligrams of sub-fraction B2-3 was purified by column chromatography (Sephadex LH-20, MeOH; silica gel, $CHCl_3$/MeOH, 5:1, v/v) to obtain crustecdysone (**8**, 39.0 mg). One hundred and twenty milligrams of sub-fraction B2-5 was separated by semi-preparative HPLC (Zorbax SB-C18 column; MeCN/H_2O, 50:50, 2 mL/min) to obtain pariposide A (**7**, 4.5 mg, t_R=12.788 min), formosanin C (**2**, 1.4 mg, t_R=14.634 min), dioscin (**3**, 14.4 mg, t_R=18.070 min), polyphyllin D (**1**, 12.7 mg, t_R=21.019 min), and diosgenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**4**, 4.4 mg, t_R=26.029 min). One hundred and thirty-two milligrams of sub-fraction B2-6 was purified by semi-preparative HPLC (Zorbax SB-C18 column; MeCN/H_2O, 40:60, 2 mL/min) to obtain pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (**6**, 4.1 mg, t_R=18.421 min) and paris saponin H (**5**, 9.0 mg, t_R=23.587

min).

2.4. In vitro assay for cytotoxic activity

The extracts and isolated compounds were tested *in vitro* for their cytotoxicity against the proliferation of human leukemia HL-60, human liver cancer SMMC-7721, human lung cancer A-549, human breast cancer MCF-7, and human rectal cancer SW480 cell lines using a 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2*H*-tetrazolium (MTS; Promega, Beijing, China) assay. These cell lines were obtained from ATCC (Manassas, VA, USA).

The MTS assay was performed as previously described (Mosmann, 1983; Yang et al., 2013). In general, 100 μL of a suspension of cells in the log phase of their cycle were seeded into 96-well plates (5000–10000 cells/well, NEST Biotechnology, Wuxi, China), with the cell suspension containing 10% fetal bovine serum (DMEM or RPMI1640, Thermo Fisher Scientific, Beijing, China). After 12 h of incubation at 37 °C, each sample to be tested was added. The cancer cell lines were exposed to the test extracts or compounds at five different concentrations, each in triplicate. After incubation for 48 h at 37 ºC, an MTS (20 μL) solution and DMEM (100 μL) were added into the well. The incubation was continued for another 1–4 h. The absorbance was measured at the detection wavelength of 490 nm (L_1) and the reference wavelength of 680 nm (L_2), and cytotoxicity was expressed as IC_{50} values (Reed and Muench, 1938). Cisplatin (Sigma) was used as a positive control.

2.5. In vivo antitumor activity of the total saponins from P. forrestii by intraperitoneal administration

H22 tumor cells were provided by the School of Pharmaceutical Sciences & the Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University. Kunming mice (female; weight: 18–22 g) were provided by Kunming Chu Shang Technology Co. Ltd [License No. SCXK (Xiang) 2013-0004]. H22 cells were maintained in the ascitic form by sequential passages into the mice by means of intraperitoneal (ip) transplantation of 2×10^7 cells/mouse every 5–7 days. At the end of the 2nd passage, ascites were extracted and diluted with normal saline (N.S) to 5×10^7 cells per mL (Yi et al., 2016). Diluted ascites (0.2 mL) were injected into the right armpit of each mouse (90 mice in total) to establish a xenograft model. After 24 h, the mice were fasted for 4 h and randomly divided into 6 groups. The total saponins were diluted in a 0.5% sodium carboxymethyl cellulose solution (0.5% CMC-Na). The dosages administered were 0.56 mg/kg, 1.13 mg/kg, and 2.25 mg/kg. Additionally, 0.5% CMC-Na and N.S were used as vehicle controls and cisplatin (2 mg/kg, diluted in N.S) was used as a positive control. All of the samples were administered ip at 0.2 mL/10 g once per day for 7 days. After 7 days of administration and subsequent withdrawal for 1 day, the mice were sacrificed and their body, tumor, liver, kidney, spleen and thymus weights were measured. Organ coefficient (mg/g)=organ weight/body weight (Jin et al., 2017). Rates of inhibition of tumors, body weights, and organs were calculated by the following equations: inhibition rate (%)=(A−B)/A×100% (A is the average weight or organ coefficient of the vehicle control group and B is that of the treated group).

All data are presented as the mean±standard deviation (SD). The differences

between groups were evaluated using one way ANOVA. The mean values were considered to be statistically significant at a *P* value <0.05. Statistical analyses were carried out using Statistic Pocket for Social Science (SPSS) version 16.0.

3. Results and discussion

3.1. Structure elucidation

The compounds (**1**–**8**) (Figure 1) isolated from the rhizomes of *P. forrestii* were elucidated to be polyphyllin D (**1**) (Chen and Zhou, 1987), formosanin C (**2**) (Munday et al., 1993), dioscin (**3**) (Wang et al., 2001), diosgenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**4**) (Aydin et al., 2014; Chen et al., 1983), paris saponin H (**5**) (Pang et al., 2015), pennogenin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (**6**) (Nakano et al., 1989), pariposide A (**7**) (Wu et al., 2012), and crustecdysone (**8**) (Nishimoto et al., 1987) by comparison of their NMR and MS data with those of known compounds in the literature.

The aerial parts of *Paris* plants are also sources of steroidal saponins. We prepared formosanin C and paris saponin VII from the pericarps of *P. polyphylla* var. *yunnanensis* (Wang et al., 2017a). Further research needs to be conducted on the chemical constituents in the aerial parts of *P. forrestii*.

3.2. In vitro cytotoxic activity

As shown in Table 1, the crude extracts, EtOAc-soluble fraction, and *n*-butanol-soluble fraction (total saponins) from *P. forrestii* possessed growth-inhibitory activity against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines. Among these extracts, total saponins were most active against the five cancer cell lines, with IC_{50} values of 1.66 μg/mL, 2.09 μg/mL, 0.45 μg/mL, 0.50 μg/mL, and 0.60 μg/mL. Therefore, the chemical constituents of the total saponins

were further isolated and identified. Seven saponins (**1**–**7**) and one steroid crustecdysone (**8**) were obtained. Saponins **1**–**6** showed significant growth-inhibitory activity against the HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines, with IC_{50} values from 0.58 μM to 11.04 μM, while saponin **7** only weakly inhibited the A-549 and SW480 cell lines, with IC_{50} values of 20.28 μM and 40.11 μM, respectively. The steroid crustecdysone was inactive (IC_{50}>40 μM).

Polyphyllin D (**1**), formosanin C (**2**), polyphyllin VI (**9**), and paris saponin VII (**10**) (Figure 1) were used as standard compounds to assess the quality of Chong-lou according to the Chinese Pharmacopoeia. Compounds **9** and **10** were purchased and also evaluated for cytotoxicity in the present study. These two saponins were toxic against the five cancer cell lines, with IC_{50} values from 2.84 μM to 6.29 μM (Table 1).

Comparing the activity data between compounds **4** and **7**, it was determined that the formation of a 5,8-peroxide bridge reduces the activity of this type of saponin. The activity of diosgenin-3-*O*-triglycosides (**1** and **3**) seems to be higher than that of diosgenin-3-*O*-diglycoside (**4**) and diosgenin-3-*O*-tetraglycoside (**2**), as determined by comparison of the data from compounds **1** and **3** with those from compounds **2** and **4**. A previous review mentioned that diosgenyl saponins had more strongly cytotoxic activities than did pennogenyl saponins (Man et al., 2013). However, the present data (Table 1) did not support this view by comparison of the data from saponins **1**–**4** with those from saponins **5**, **6**, **9**, and **10**.

3.3. The total saponins from P. forrestii inhibited H22 tumor growth through intraperitoneal administration in mice

Samples were administered in mice for 7 days. Their body, tumor, liver, kidney, spleen and thymus weights were measured after sacrifice. The tumor inhibitory rate is shown in Table 2. Compared with the inhibition rates of the control 0.5% CMC-Na,

the tumor inhibitory rates of the low, middle and high dosages (0.56, 1.13, and 2.25 mg/kg) of the total saponins were 18.9%, 14.9% and 42.6% (P<0.01), respectively. The high dosage of the total saponins significantly inhibited the growth of the tumor, much stronger than the middle dosage (P<0.05). Compared with the inhibitory activity of N.S, cisplatin significantly inhibited the growth of tumors by ip administration (2 mg/kg dose), with an inhibitory rate of 53.9% (P<0.01).

In addition to tumor inhibition, the inhibition rates of the body weight and organ coefficient were measured to evaluate the toxicity of the samples (Tables 3–5). The inhibition rates of the high dosage of total saponins for the body weight, liver and thymus in mice were 26.0% (P<0.0.1), 17.3% (P<0.01) and 80.3% (P<0.01), respectively. For the kidney and spleen, no obvious inhibitory effects were found from total saponins, though the high dosage of the total saponins increased the kidney coefficient by 12% (P<0.05) and the middle dosage increased the spleen coefficient by 36.6% (P<0.01). However, the inhibitory rates of cisplatin for the body weight, liver, spleen and thymus were 23.9% (P<0.01), 16.4% (P<0.01), 59.4% (P<0.01) and 53.7% (P<0.01), respectively. These results indicate that total saponins had a weaker inhibitory effect on the mouse spleen, while they had a stronger inhibitory effect on the mouse thymus than cisplatin upon ip administration.

4. Conclusion

Saponins, with potent anticancer activities both *in vitro* and *in vivo*, represent the active fractions of extracts from *P. forrestii* rhizomes. Steroidal saponins **1**–**6** are the major cytotoxic constituents of the total saponins. The results support that *P. forrestii* could be a substitute for *P. polyphylla* var. *yunnanensis* as an anticancer medicine.

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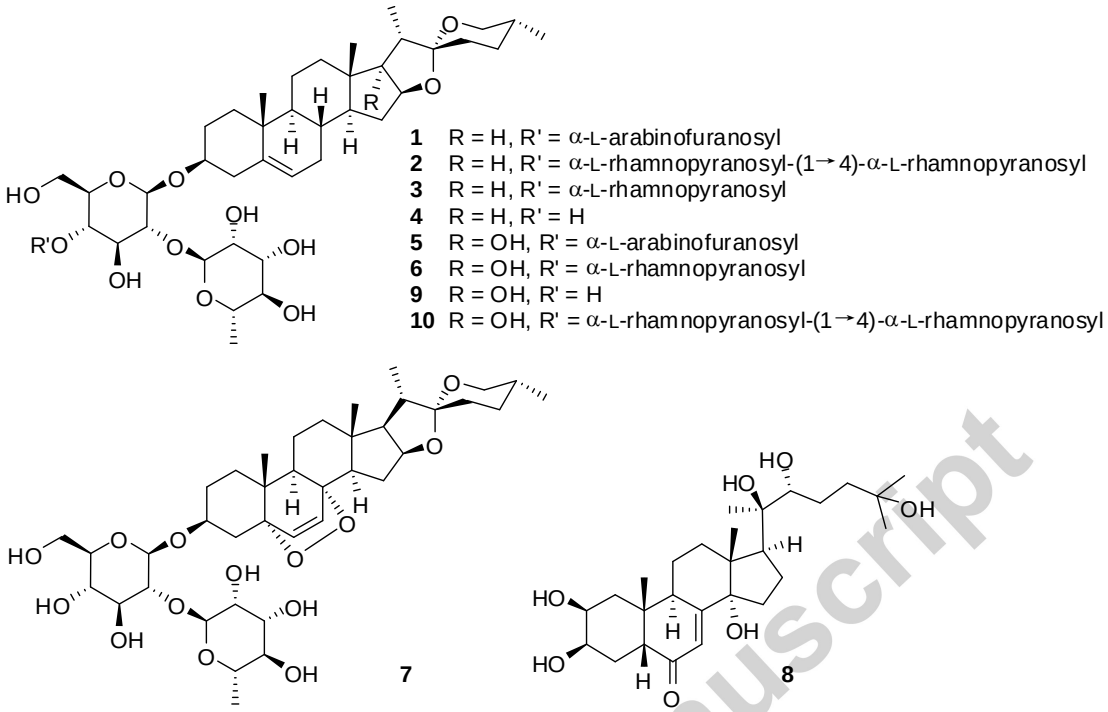

Fig. 1. Structures of compounds **1**–**8** from *Paris forrestii*, along with **9** and **10** obtained by purchasing.

Table 1 IC_{50} values of extracts and compounds (**1**–**8**) from *Paris forrestii* against five cancer cell lines.

Extracts and compounds	IC_{50}, μg/mL (μM) [a]				
	HL-60	SMMC-7721	A-549	MCF-7	SW480
Crude extracts	33.18	40.52	9.24	46.84	37.31
EtOAc-soluble fraction	22.78	24.75	8.06	27.87	10.32
n-BuOH-soluble fraction (total saponins)	1.66	2.09	0.45	0.50	0.60
1	0.90 (1.05)	0.52 (0.61)	1.29 (1.51)	0.62 (0.72)	0.58 (0.68)
2	11.21 (11.04)	2.78 (2.74)	2.99 (2.95)	2.16 (2.13)	1.08 (1.06)
3	2.95 (3.39)	0.69 (0.79)	0.98 (1.13)	0.56 (0.64)	0.50 (0.58)
4	7.29 (10.08)	2.19 (3.03)	2.26 (3.12)	2.55 (3.53)	2.30 (3.18)
5	2.58 (2.96)	2.72 (3.12)	0.71 (0.82)	3.07 (3.52)	2.38 (2.73)

6	0.91 (1.03)	1.04 (1.18)	0.62 (0.70)	2.07 (2.34)	0.67 (0.76)
7 [b]	INA	INA	15.28 (20.29)	INA	30.20 (40.11)
8 [b]	INA	INA	INA	INA	INA
9 [c]	2.59 (3.50)	2.53 (3.42)	2.59 (3.50)	4.65 (6.29)	2.77 (3.75)
10 [c]	3.44 (3.34)	3.64 (3.53)	2.93 (2.84)	3.79 (3.68)	3.45 (3.35)
Cisplatin (positive control)	0.11 (0.37)	0.64 (2.14)	0.57 (1.89)	1.59 (5.30)	1.22 (4.05)

[a] The concentration unit for data in parentheses is μM.

[b] INA=inactive, IC_{50}>40 μM.

[c] Compounds **9** and **10** were bought from Kunming Huangbao Trading Co., Ltd.

Table 2 Tumor inhibitory rate of the total saponins from *P. forrestii* through intraperitoneal administration in H22 tumor-bearing mice.

Group	**Dose** (mg/kg)	**Mice**		**Tumor weight** (g)	**Inhibition rate** (%)
		Start	End		
Vehicle control	0.5% CMC-Na	15	15	1.48±0.69	—
Total saponins	0.56	15	13	1.20±0.44	18.9
	1.13	15	13	1.26±0.35	14.9
	2.25	15	14	0.85±0.23[a,c]	42.6
Vehicle control	N.S	15	15	1.30±0.60	—
Cisplatin	2	15	15	0.60±0.32[b]	53.9

[a] P<0.05, compared with 0.5% CMC-Na.

[b] P<0.01, compared with N.S.

[c] P<0.05, compared with the middle dosage (1.13 mg/kg) of total saponins.

Table 3 Body weight inhibitory rate of the total saponins from *P. forrestii* through intraperitoneal administration in H22 tumor-bearing mice.

Group	**Dose** (mg/kg)	**Initial weight** (g)	**Final weight** (g)	**Initial weight/Final weight**	**Inhibition rate** (%)
Vehicle control	0.5% CMC-Na	22.67±2.01	32.94±4.35	1.46±0.16	—
Total saponins	0.56	22.09±2.37	31.33±4.32	1.42±0.17	2.7
	1.13	21.91±1.72	30.68±2.22	1.40±0.11	4.1
	2.25	21.88±1.90	23.54±2.77	1.08±0.16[a]	26.0
Vehicle control	N.S	21.70±2.20	33.30±1.80	1.55±0.14	—
Cisplatin	2	22.33±2.13	26.05±2.91	1.18±0.18[b]	23.9

[a] $P<0.01$, compared with 0.5% CMC-Na.

[b] $P<0.01$, compared with N.S.

Table 4 Liver and kidney inhibitory rate of the total saponins from *P. forrestii* through intraperitoneal administration in H22 tumor-bearing mice.

Group	**Dose** (mg/kg)	**Liver coefficient (**mg/g)	**Liver inhibition rate** (%)	**Kidney coefficient** (mg/g)	**Kidney inhibition rate (%)**
Vehicle control	0.5% CMC-Na	70.30±5.44	—	11.33±1.56	—
Total saponins	0.56	64.31±9.42	8.5	11.33±1.74	0.0
	1.13	69.70±12.32	0.9	12.12±1.26	–7.0
	2.25	58.15±7.63[b]	17.3	12.69±1.99[a]	–12.0
Vehicle control	N.S	69.20±3.97	—	11.44±0.84	—

Cisplatin	2	57.86±10.28[c]	16.4	10.73±1.27	6.2

[a] $P<0.05$, [b] $P<0.01$, compared with 0.5% CMC-Na.

[c] $P<0.01$, compared with N.S.

Table 5 Spleen and thymus inhibitory rate of the total saponins from *P. forrestii* through intraperitoneal administration in H22 tumor-bearing mice.

Group	**Dose** (mg/kg)	**Spleen coefficient** (mg/g)	**Spleen inhibition rate** (%)	**Thymus coefficient** (mg/g)	**Thymus inhibition rate** (%)
Vehicle control	0.5% CMC-Na	6.26±2.75	—	2.74±0.64	—
Total saponins	0.56	7.87±2.70	–25.7	1.18±0.74[a]	56.9
	1.13	8.55±1.41[a]	–36.6	1.92±0.76[a]	29.9
	2.25	6.85±2.41	–9.4	0.54±0.35[a]	80.3
Vehicle control	N.S	5.77±1.69	—	3.48±0.46	—
Cisplatin	2	2.34±0.55[b]	59.4	1.61±0.87[b]	53.7

[a] $P<0.01$, compared with 0.5% CMC-Na.

[b] $P<0.01$, compared with N.S.