Medicinal Plant 2016, 7(11 - 12): 56 - 59

The Effects of Alcohol and Water Extracts of *Allium wallichii* Kunth on Transplanted Hepatoma, Lung Cancer and Breast Cancer of Mice

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Abstract [**Objectives**] To study the anti-tumor effects of alcohol and water extracts of *A. wallichii* Kunth. [**Methods**] In vivo experiment of transplanted tumors was employed to observe the effects of different solvent extracts of *A. wallichii* Kunth on transplanted S180 sarcoma and transplanted H22 hepatoma of KM mice, transplanted Lewis lung cancer of C57BL mice and transplanted EMT-6 breast cancer of BALB/C mice. 20 g/kg of different solvent extracts of *A. wallichii* Kunth were injected in mice with different types of transplanted tumors, while the blank control group was treated with same-dosage normal saline. After 11 days, the mice were killed, and the inhibitory rate of tumor was calculated. [**Results**] Compared with blank control group, the tumor weight of medicated group decreased obviously ($P<0.05$ or $P<0.01$), and the group treated with the extracts of roots and rhizomes, and leaves of *A. wallichii* Kunth had more obvious decrease effect in the same dosage ($P<0.05$ or $P<0.01$). [**Conclusions**] Different solvent extracts of *A. wallichii* Kunth showed anti-tumor effect, especially the extracts of roots and rhizomes, leaves of *A. wallichii* Kunth showed the obvious effect.

Key words *A. wallichii* Kunth, Anti-tumor activity, Hepatoma, Lung cancer, Breast cancer

1 Introduction

Allium wallichii Kunth belongs to *Allium*, Liliaceae, is one of sources of Yi medicine Luowomo, and is commonly used clinical medicinal herb of Yi medicine[1]. It has the effects of expelling Ascaris, easing pain and cough, spreading the cold, blood circulation, treating hemorrhoid, detumescence, and is mainly used to treat Ascaris pain, abdominal pain, bronchocephalitis, cold, traumatic injury, wound by a sword, hemorrhoid and so on[2-3]. In Yi Nationality and Miao Nationality, *A. wallichii* Kunth is also a kind of medicinal and edible plant. *Herbs of South Yunnan* recorded that *A. wallichii* Kunth could be eaten, which could nourish blood, strengthen spleen and bone, and enhance power. When it is crushed and compressed on the affected part, traumatic injury could be treated; when root is crushed with halloysite and then is dried into the powder, it could treat injury by sword or axe, with miraculous effect[4-5]. Researches in recent years find that *Allium* plants contain polysaccharide, sulfur compound, steroidal saponins, flavone and nitrogen compounds, and have the biological activities of resisting platelet aggregation, tumor, fungi and strengthening Yang[6-19], which has very good development prospect and economic value. In this paper, the anti-tumor effects of different solvent extracts of *A. wallichii* Kunth were studied, which aimed to provide test data and scientific reference for studying biological activity and quality evaluation of *A. wallichii* Kunth, and lay basis for safe, effective and controllable exploration and utilization of *A. wallichii* Kunth.

Received: September 22, 2016 Accepted: November 3, 2016
Supported by National Science and Technology Support Plan (2015BAC-05B02); Sichuan Science and Technology Support Plan (2015SZ0034); Education and Teaching Research and Innovation Item of Southwest University for Nationalities (2015).

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2 Materials and methods

2.1 Materials *A. wallichii* Kunth was collected from Dajiucaiping of Hezhang County, Guizhou in 2012. Via identification of Professor Liu Yuan from Southwest University for Nationalities, it was herb of *A. wallichii* Kunth, and the sample was conserved in Ethnic Medicine Institute, Southwest University for Nationalities. There were BALB/C mice and C57BL mice, with 6 - 8 weeks old, 18 - 22 g, all male, and they were bought from Experimental Animal Center of Sichuan University and were all SPF-level qualified animals. There were 80 Kunming mice, with 20 - 24 g, half male and half female, and they were all provided by Experimental Animal Center of Sichuan Academy of Chinese Medicine Sciences. EMT-6 tumor strain and Lewis tumor strain were provided by Research Laboratory of Experimental Tumor, West China Center of Medical Sciences, Sichuan University, while H22 tumor strain and S180 tumor strain were provided by Institute of Pharmacology Toxicology, Sichuan Academy of Chinese Medicine Sciences.

2.2 Reagents Cisplatin for injection (Qilu Pharmaceutical Co., Ltd.), batch number: 1110061DV; cyclophosphamide for injection(Tonghua Maoxiang Pharmaceutical Co., Ltd.), batch number: 130104; sodium chloride injection (Kunming Nanjiang Pharmaceutical Co. Ltd), batch number: C130814A. Dimethyl sulfoxide and 95% ethanol, *etc.* were all AR, and water was twice distilled water.

2.3 Instruments JA1003 type of plate electronic balance (Shanghai Jingke Balance Factory); YB1201 type of electronic

balance (Shanghai Haikang Electronic Instrument Factory); 0.25 mL of full-glass syringe with blue core (Shanghai Medical Laser Instrument Factory).

2.4 Preparation of extract Ethanol extract: the collected fresh *A. wallichii* Kunth was washed by flow clean water, and then it was divided into three parts: root and rhizome, leaf, flower and fruit. Each part was cut, and 95% of ethanol was added according to the material liquid ratio of 1∶20 (g∶mL). After soaked for 24 h, it was filtrated, and the filtrate was depressurized and concentrated into extractum. The extract was collected, and ethanol was recovered. 95% of recovered ethanol with the same volume was added in filter residue, and above operation was repeated. Each part of *A. wallichii* Kunth was extracted for three times, and the extracts were merged.

Water extract: filter residue after ethanol extraction was recovered, and set for ventilation and airing until no ethanol smell. Appropriate amount of dry medicinal material from each part was weighed. According to material liquid ratio of 1∶20 (g∶mL), distilled water was added to soak for 30 min. It was heated by small fire, and micro boiling was kept for 30 min. After that, it was set for cooling to room temperature, and then filtrated, and the filtrate was collected. Filter residue continued to repeat the above operation twice, and three times of filtrate was merged. Filtrate was depressurized and concentrated into extractum, and extract was collected. The obtained ethanol and water extracts of *A. wallichii* Kunth each part were dried into powder under low temperature freezing conditions, and then extracts of each part were obtained, and results were shown as Table 1.

2.5 Medicine preparation Appropriate amount of *A. wallichii* Kunth extract was taken, and equal amount of dimethyl sulfoxide was added. After mixed evenly, appropriate amount of normal saline was added, thereby making 10% of *A. wallichii* Kunth extract emulsion liquid. Before using it, normal saline was added to dilute into suitable concentration.

Table1 The yields of extracts from different parts of *A. wallichii* Kunth

Extraction method	Position	Extractive yield//%	No.
Aqueous	Roots and rhizomes	8.02	A1
	Leaves	0.71	A2
	Flowers and fruits	1.72	A3
Alcohol	Roots and rhizomes	0.92	B4
	Leaves	1.88	B5
	Flowers and fruits	2.28	B6

3 Results and analyses

3.1 Impact of *A. wallichii* Kunth extract on transplanted S180 of KM mice 80 mice with the weight of 18 – 22 g were taken, and there were 10 mice in each group. After suitable for environment for 4 d in the laboratory, S180 ascites inoculated in KM mice for 10 days was taken to make into tumor cell suspension. The number of S180 living cell was adjusted into 1×10^6 cells/mL, and the dosage was 0.2 mL/mouse. The S180 cells were injected in axillary position of left forelimb. After inoculated for 24 h, mice inoculated tumor strain were randomly divided into model group and dosing group. Animals of model group were fed by normal saline, and dosage of dosing group was shown as Table 2. After dosing medicine for 10 d, mice were killed on the 11th day to detect the weight of tumor, and data were shown by $\bar{x} \pm s$. t test was used to detect statistical difference between dosing group and model group, and inhibition rate of tumor was calculated. Calculation formula of tumor inhibition rate was as below: The turor control rate(%) $= \frac{C-T}{T} \times 100$. Here, T was average tumor weight of treatment group, while C was average tumor weight of normal saline control group, and results were shown as Table 2.

Table 2 The effects of *A. wallichii* Kunth extractives on S180 sarcoma of KM mice

Group	Drug//g/kg	Body mass//g		Tumor mass//g	Inhibitory rate//%
		Initial	Final		
Normal control	0	22.5 ± 1.4	37.0 ± 2.2	2.39 ± 0.91	/
Cyclophospha	0.02	23.0 ± 1.5	30.9 ± 1.6*	0.87 ± 0.36**	63.4
A1	20	22.9 ± 1.3	34.9 ± 4.5	1.23 ± 1.13*	48.4
A2	20	22.6 ± 1.2	36.0 ± 5.0	1.46 ± 0.67*	38.7
A3	20	22.4 ± 1.1	37.4 ± 5.8	1.69 ± 0.86	29.3
B4	20	24.1 ± 2.2	35.1 ± 3.6	1.08 ± 0.66**	54.9
B5	20	23.5 ± 2.3	35.9 ± 4.2	1.31 ± 0.97*	45.2
B6	20	23.0 ± 1.7	34.7 ± 6.2	1.83 ± 1.05	23.4

Note: * $P<0.05$, ** $P<0.01$.

Test results showed that tumor weights of each dosing group by water and alcohol extracts of root and rhizome, leaf of *A. wallichii* Kunth obviously decreased when compared with model group ($P<0.05$ or $P<0.01$), which had better inhibition on transplanted S180 sarcoma.

3.2 Impact of *A. wallichii* Kunth extract on transplanted H22 of KM mice 80 mice with the weight of 18 – 22 g were taken, and there were 10 mice in each group. After suitable for environment for 4 d in the laboratory, H22 ascites inoculated in KM mice for 10 days was taken to make into tumor cell suspension. The number of H22 living cell was adjusted into 1×10^6 cells/mL, and the dosage was 0.2 mL/mouse. The H22 cells were injected in axillary position of left forelimb. After inoculated for 24 h, mice inoculated tumor strain were randomly divided into model group and dosing group. Animals of model group were fed by normal saline, and dosage of dosing group was shown as Table

3. After dosing medicine for 10 d, mice were killed on the 11th day to detect the weight of tumor, and data were shown by $\bar{x} \pm s$. t test was used to detect statistical difference between dosing group and model group, and inhibition rate of tumor was calculated (Table 3).

Table 3 The effects of *A. wallichii* Kunth extractives on transplanted H22 hepatoma of KM mice

Group	Drug//g/kg	Body mass//g		Tumor mass//g	Inhibitory rate//%
		Initial	Final	$\bar{x} \pm s$	
Normal control	0	27.9 ±1.4	37.9 ±3.2	1.31 ±0.52	/
Cyclophospha	0.02	29.0 ±2.7	34.9 ±4.2	0.73 ±0.27**	44.3
A1	20	27.4 ±2.3	32.7 ±5.5*	0.63 ±0.39**	52.2
A2	20	27.1 ±2.1	36.3 ±5.1	0.79 ±0.47*	39.3
A3	20	27.3 ±2.3	34.6 ±3.8*	0.95 ±0.45	27.7
B4	20	27.2 ±2.0	35.0 ±5.0	0.75 ±0.38*	42.6
B5	20	27.4 ±1.8	37.2 ±3.5	0.96 ±0.40	26.6
B6	20	28.1 ±1.8	37.1 ±5.1	0.89 ±0.49	32.1

Note: * $P<0.05$, ** $P<0.01$.

Test results showed that tumor weights of each dosing group by water extracts of root and rhizome, leaf of *A. wallichii* Kunth and alcohol extracts of root and rhizome of *A. wallichii* Kunth obviously decreased when compared with model group ($P<0.05$ or $P<0.01$), which had better inhibition on transplanted H22 hepatoma; alcohol extracts of *A. wallichii* Kunth flower and fruit also had certain inhibition on H22 hepatoma.

3.3 Impact of *A. wallichii* Kunth extract on transplanted Lewis lung cancer of C57BL mice 80 C57BL mice were taken, and there were 10 mice in each group. After suitable for environment for 7 d in the laboratory, several C57BL mice with good tumor growth (Lewis lung cancer) were killed, and tumor was taken for weighing. The sterilized normal saline which was three times of tumor weight [1∶3 (W/V)] was added to make cancer cell suspension, and 0.2 mL of cancer cell suspension was inoculated in each mouse. After inoculated for 24 h, mice inoculated tumor strain were randomly divided into model group and dosing group. Animals of model group were fed by normal saline, and dosage of dosing group was shown as Table 4. After dosing medicine for 12 d, mice were killed to detect the weight of tumor, and data were shown by $\bar{x} \pm s$ (Table 4).

Table 4 The effects of *A. wallichii* Kunth extractives on transplanted Lewis lung cancer of C57BL mice

Group	Drug//g/kg	Body mass//g		Tumor mass//g	Inhibitory rate//%
		Initial	Final	$\bar{x} \pm s$	
Normal control	0	20.0 ±0.6	21.6 ±1.0	1.55 ±0.51	/
Cisplatin	0.001	20.0 ±0.7	20.9 ±1.2	0.84 ±0.40**	45.9
A1	20	20.0 ±0.4	21.8 ±0.9	1.14 ±0.25*	26.5
A2	20	20.1 ±0.6	22.1 ±1.2	1.38 ±0.37	11.5
A3	20	20.1 ±0.7	21.9 ±0.6	1.27 ±0.27	18.0
B4	20	19.9 ±0.7	21.5 ±1.0	0.95 ±0.26**	39.0
B5	20	20.0 ±0.7	22.3 ±0.9	1.02 ±0.26**	34.1
B6	20	19.7 ±0.6	21.8 ±0.9	1.16 ±0.40	25.3

Note: * $P<0.05$, ** $P<0.01$.

Table 5 The effects of *A. wallichii* Kunth extractives on transplanted EMT-6 breast cancer of BALB/C mice

Group	Drug//g/kg	Body mass//g		Tumor mass//g	Inhibitory rate//%
		Initial	Final		
Normal control	0	20.4 ±0.3	23.1 ±1.3	1.31 ±0.30	/
Cisplatin	0.001	20.3 ±0.8	22.8 ±1.1	0.66 ±0.36**	49.5
A1	20	20.0 ±0.5	22.6 ±1.2	0.81 ±0.28**	37.8
A2	20	20.2 ±0.6	22.6 ±1.0	1.15 ±0.26	11.9
A3	20	20.1 ±0.7	22.9 ±1.0	1.23 ±0.42	6.1
B4	20	20.0 ±0.7	23.1 ±1.2	0.73 ±0.29**	43.9
B5	20	20.1 ±0.6	23.1 ±1.3	0.90 ±0.43*	30.7
B6	20	19.9 ±0.6	22.6 ±1.0	0.86 ±0.29**	33.9

Note: * $P<0.05$, ** $P<0.01$.

Test results showed that tumor weights of each dosing group by water extracts of root and rhizome of *A. wallichii* Kunth and al-

cohol extracts of root, rhizome and leaf of *A. wallichii* Kunth obviously decreased when compared with model group ($P<0.05$ or $P<0.01$), which all had better inhibition on transplanted Lewis lung cancer.

3.4 Impact of of *A. wallichii* Kunth extract on transplanted EMT-6 breast cancer of BALB/C mice 80 BALB/C mice were taken, and there were 10 mice in each group. After suitable for environment for 7 d in the laboratory, several BALB/C mice with good tumor growth (EMT-6 breast cancer) were killed, and tumor was taken for weighing. The sterilized normal saline which was three times of tumor weight [1∶3 (W/V)] was added to make cancer cell suspension, and 0.2 mL of cancer cell suspension was inoculated in each mouse. After inoculated for 24 h, mice inoculated tumor strain were randomly divided into model group and dosing group. Animals of model group were fed by normal saline, and dosage of dosing group was shown as Table 5. After dosing medicine for 11 d, mice were killed to detect the weight of tumor, and data were shown by $\bar{x} \pm s$ (Table 5).

Test results showed that tumor weights of each dosing group by water extracts of root and rhizome of *A. wallichii* Kunth and alcohol extracts of root and rhizome, leaf, flower and fruit of *A. wallichii* Kunth obviously decreased when compared with model group ($P<0.05$ or $P<0.01$), which all had better inhibition on transplanted EMT-6 breast cancer.

4 Discussions

The selection of tumor model is an important link of the drug resisting tumor test. To avoid false positive, false negative and different sensitivities of drug to different tumor models, transplanted tumor model of the same kind of animal should select three kinds of mouse transplanted tumors or more for experiment, to treat solid tumor and observe inhibition rate of tumor weight[20]. In this paper, tumor cell models of S180 sarcoma, H22 hepatoma, Lewis lung cancer and EMT-6 breast cancer were used to evaluate alcohol and water extracts of different parts from *A. wallichii* Kunth. Test results showed that for different transplanted tumors, antitumor activity of alcohol extract was stronger than that of water extract. In the treatment tests of extracts from different parts of *A. wallichii* Kunth, extracts of root and rhizome, leaf had stronger anti-tumor activities on transplanted S180 sarcoma, H22 hepatoma, Lewis lung cancer; alcohol extracts of flower and fruit had obvious inhibition on EMT-6 breast cancer ($P<0.01$); water extracts of flower and fruit from *A. wallichii* Kunth had insignificant anti-tumor effect on the four kinds of tumor models ($P>0.05$). Overall, alcohol and water extracts of *A. wallichii* Kunth all had certain inhibition on transplanted hepatoma, lung cancer and breast cancer, especially extracts of *A. wallichii* Kunth root and rhizome, leaf had obvious anti-tumor effects. In clinical application, it could select extracts of different parts of *A. wallichii* Kunth to treat according to different tumors. It also needs further studying content difference of active component resisting tumor in extracts of different parts from *A. wallichii* Kunth and anti-tumor action mechanism.

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