

Study on the extraction of flavonoids from *Artemisia integrifolia* and its anti-lung cancer activity

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Abstract: Objective: To extract flavonoids from *Artemisia integrifolia* and determine their content, to evaluate their activity. Method: Total flavonoids were extracted using ultrasonic extraction, and the inhibitory effect on the proliferation of lung cancer cells H1299 was investigated. Additionally, a preliminary study was conducted on the treatment of liver degeneration. Results: Using a 50% ethanol solution, ultrasonic treatment at 90°C for 50 minutes followed by reflux for 3 hours resulted in the highest total flavonoid extraction rate. The study showed that it has a certain inhibitory effect on the proliferation of lung cancer H1299 cells, with an IC50 value of approximately 200µg/mL measured by MTT assay. The treatment also showed therapeutic effects on liver degeneration.

1. Introduction

Artemisia integrifolia, commonly known as willow leaf mugwort, water mugwort, and white mugwort, is a perennial herb belonging to the genus *Artemisia* of the family Asteraceae [1-3]. The resources of *Artemisia integrifolia* along the banks of the Nen River in Qiqihar are abundant, making it a pure, natural, wild edible wild vegetable. Willow herb stems contain high levels of flavonoids, which have good therapeutic effects on lowering blood pressure, reducing blood lipids, and alleviating cardiovascular diseases[4-7]. Moreover, it also shows potential in cancer prevention and tumor inhibition. This paper extracts total flavonoids using ultrasonic extraction and investigates their inhibitory effect on lung cancer cell proliferation. This will provide theoretical basis for the development and utilization of *Artemisia integrifolia* and further research into value-added products of *Artemisia integrifolia*, contributing to the development of local resources and the creation of functional foods that meet people's needs.

2. Materials and methods

2.1. Main reagents

Luteolin standard (Aladdin Biochemical Technology Co., Ltd.); anhydrous ethanol, sodium hydroxide, aluminum nitrate, sodium nitrite, all of analytical grade; dried *Artemisia integrifolia*: (collected locally); fetal bovine serum (Jiangsu Kejing Biotechnology Co., Ltd.); DMEM culture medium, double antibody (Thermo Fisher

Scientific Co., Ltd.); lung cancer cell H1299 (Shanghai Baililai Biotechnology Co., Ltd.)

2.2. Main Instruments

High-speed freezing centrifuge (Sichuan Shuke Instrument Co., Ltd.), CO₂ incubator (Shenzhen Ruiwode Life Technology Co., Ltd.); ELISA reader (Thermo Fisher Scientific Co., Ltd.); fluorescence microscope (Guangzhou Haoyang Marine Technology Co., Ltd.); KQ-600V ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd.)

3. Experiment

3.1. Extraction of flavonoids

Weigh 10.0g pre-treated crude powder of *Artemisia integrifolia* into a 1L beaker, add 400mL of distilled water (material ratio 1:40). Soak for a few minutes, then place in an ultrasonic cleaner for 50 minutes. Transfer to a water bath and extract at a constant temperature of 90°C for 3 hours. While still hot, filter and discard the residue. Cool to 60-70°C, adjust to pH 4-5 with concentrated hydrochloric acid, and let stand for one hour until a solid precipitates. Place the solid in distilled water, heat to boiling for 15 minutes, then filter while still hot, discard any residue, and allow to stand completely. Filter again, collect the crystals, and dry them in an oven at 70°C to obtain total flavonoids.

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3.2. Determination of flavonoid content in willow sprout

Weigh 0.040 g of rutin standard to a 50mL volumetric flask, dissolve with an appropriate amount of 50% ethanol solution, shake well, and adjust to the mark. Then, transfer 0.0mL, 1.0mL, 2.0mL, 3.0mL, 4.0mL, 5.0mL of the rutin standard solution into a 25 mL volumetric flask, add 1 mL of 5% NaNO₂ and 1 mL of 10% Al(NO₃)₃ solution each time, shake well, let stand for 6 minutes, then add 10 mL of 4% NaOH solution, and adjust to the mark with 50% ethanol solution to 50 mL. Let stand for 15 minutes, then measure the absorbance at 510 nm. Prepare an appropriate concentration of flavonoid sample solution in the same manner to measure its absorbance. Plot the absorbance against the standard curve to calculate the concentration and yield.

3.3. Inhibition of cell proliferation by liuhaoyao flavonoid

Cultured lung cancer cells H1299 were propagated to the second generation, and the proliferation inhibition of the second-generation lung cancer cells H1299 was investigated using *Artemisia integrifolia* flavonoid. Different concentrations of *Artemisia integrifolia* flavonoid solution, including 800µg/mL, 400µg/mL, 200µg/mL, 100µg/mL, 50µg/mL, and 25µg/mL, were prepared from the culture medium. After culturing for 24 hours, 20 microliters of 5mg/mL MTT solution was added to each well, and the cells were cultured at 37°C for four hours. The supernatant was discarded, and 150 microliters of DMSO solution was added to each well, followed by shaking for 10 minutes to ensure complete dissolution of the crystals. The absorbance values were measured at 490nm using a microplate reader to evaluate the inhibitory effects of different concentrations of flavonoids on the cells.

3.4 Preliminary study on the effect of Liriope flavonoid on the pathological changes of liver injury in rats with hepatic lipidation

A 4-week CCl₄ olive oil suspension was administered to rats, causing liver damage with fatty and inflammatory changes, successfully modeling the condition. The study included a control group (normal feeding without modeling), a placebo group, and a treatment group. The placebo group received distilled water, while the treatment group received 200mg/kg of crude flavonoids, administered three times daily, 10mL each time, for 8 weeks. Liver tissue sections were examined under a light microscope to observe the pathological changes in liver tissue.

4. Results and discussion

4.1. Determination of total flavonoids in willow shoots

Standard solutions were prepared with rutin standard,

and absorbance was measured by UV spectrophotometry. The data are shown in Table 1, and the standard curve is shown in Figure 1.

Table 1. Absorbance of rutin standard solution at different concentrations

number	1	2	3	4	5	6
Quercetin concentration(mg/mL)	0.00	0.032	0.064	0.096	0.128	0.160
Absorbance (A)	0.00	0.086	0.131	0.167	0.203	0.239

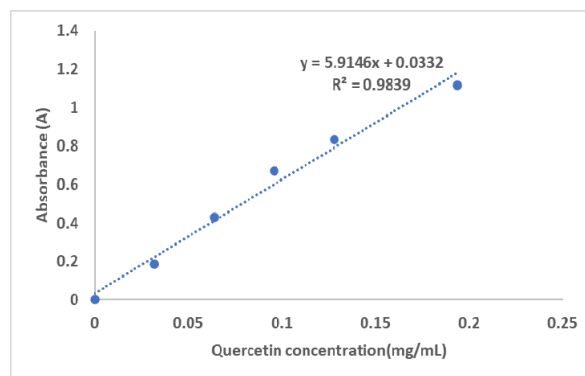


Figure 1. Rutin standard curve

The flavonoid extraction rate of ultrasonic-ethanol extraction method was determined by standard curve as 5.71%.

4.2. Study on inhibition of cell proliferation by total flavonoids from willow wormwood sprout

Fenoflavone solutions with different concentrations were prepared to investigate their inhibitory effects on H1299 lung cancer cells. The results were measured three times in parallel, as shown in Table 2.

Table 2. Inhibition rate of H1299 lung cancer cells at different concentration gradients

group	Drug concentration (µg/mL) and cell inhibition rate (%)						IC50	
	0	25	50	100	200	400		800
Group1	0	-13.45	7.64	3.27	56.91	85.82	86.18	200.07
Group2	0	-0.35	11.27	4.23	57.96	85.92	86.27	210.95
Group3	0	-15.70	11.61	5.84	61.47	85.14	84.34	199.85

As shown in Table 2, the data represent three parallel experiments to test the inhibition rate of flavonoids from *Artemisia integrifolia* on lung cancer cells, with a IC₅₀ value around 200µg/mL. When the drug concentration is 0µg/mL, the cell survival after 24h of culture is shown in Figure 2. When the drug concentration is 200µg/mL, the cell survival after 24h of culture is shown in Figure 3. When the drug concentration is 400µg/mL, the cell survival after 24h of culture is shown in Figure 4.

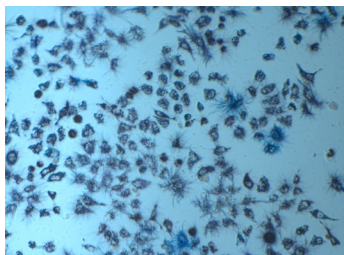


Figure 2. Flavonoid concentration 0 µg/mL

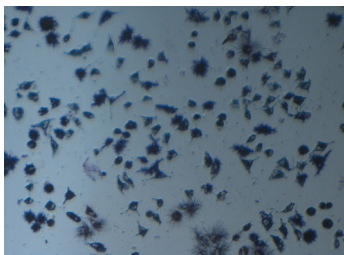


Figure 3. Flavonoid concentration 200µg/mL



Figure 4. Flavonoid concentration 400µg/mL

Figures 2, 3, and 4 show the growth of lung cancer cells under drug concentrations of 0 µg/mL, 200µg/mL, and 400µg/mL. Figure 2 indicates no inhibition, Figure 3 shows half-inhibition, and Figure 4 demonstrates complete inhibition. These results indicate that the drug has an inhibitory effect on H1299 lung cancer cells, with the inhibitory effect being directly proportional to the drug concentration. The IC₅₀ value of the drug is approximately 200µg/mL.

4.3 General effects of polysaccharides and flavonoids in willow sprouts on hepatic lipidated rats

Throughout the experiment, the blank group of rats exhibited shiny fur, quick reflexes, and a lively demeanor, with no signs of drowsiness. The rats showed strong activity, a hearty appetite, and good mental states. In contrast, during the modeling phase of the control group, treatment group 1, and treatment group 2, as the duration of the intraperitoneal injection of CCl₄ and olive oil suspension increased, the rats began to lose their fur color, their appetite declined, they became lethargic, and their weight gain slowed down. Some rats also became easily irritated. Over time, they exhibited symptoms such as stiffness and sluggish movements. Compared to the control group, after one week of oral administration of *Artemisiae Lutea* polysaccharide, the liver lipidization symptoms in all groups were relatively mild, their appetites and fur luster were better than those

in the control group, and their mental states significantly improved. As the dosage of the oral administration increased, the therapeutic effects on the rats became more pronounced. Compared to treatment group 1, after one week of oral administration of *Artemisiae Lutea* flavonoids, the rats in the sugar group had relatively poorer therapeutic effects, but their symptoms also showed some improvement.

4.4 Effects of artemisinin on the pathological changes of liver injury in rats with hepatic lipidation

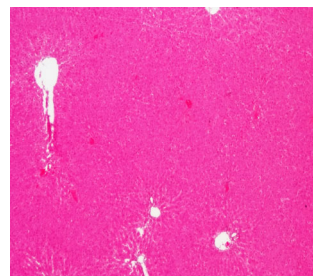


Figure 5. Blank group

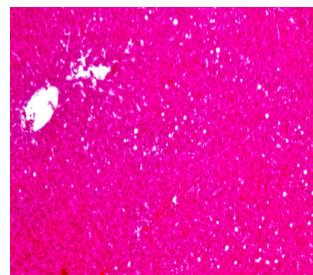


Figure 6. Medication group (200mg/kg)

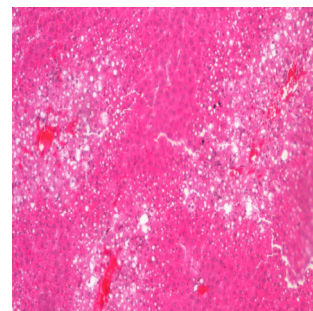


Figure 7. Low dose group of crude flavonoids (50mg/kg)

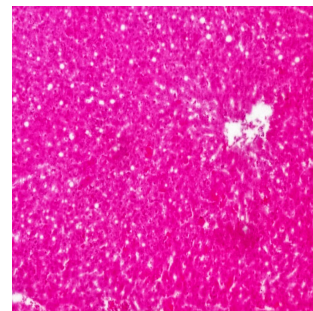


Figure 8. Medium dose group of crude flavonoids (100mg/kg)

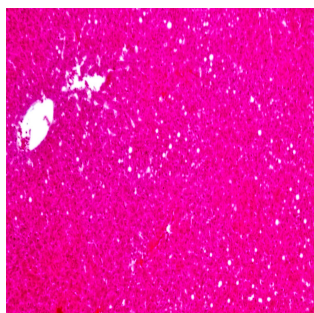


Figure 9. High dose group of crude flavonoids (200mg/kg)

HE staining was used for microscopic examination. In the normal control group, the liver structure of the rats was clear and intact, with cells arranged neatly. The hepatocytes were radially arranged around the central vein, uniform in size, and showed no pathological changes or inflammatory cell infiltration (Figure 5). In the control group, the hepatocytes were significantly swollen, with some areas showing balloon-like changes. The boundaries of the hepatic lobules were blurred, and the number of necrotic foci within the lobules increased significantly. Hepatocytes showed an increase in vacuolarization, indicating fatty degeneration, with occasional large vacuolar fatty degeneration. The portal areas also showed inflammatory cell infiltration (Figure 6). This indicates that the CCl₄ olive oil suspension administered for 4 weeks caused liver damage in rats, characterized by fatty and inflammatory pathological changes, confirming the successful establishment of the hepatic lipidation rat liver injury model. In the low-dose group (50 mg/kg) of Luteolin (a flavonoid), there was no significant improvement in the hepatocyte tissue (Figure 7); in the medium-dose group (100 mg/kg), the liver structure showed mild disarray, and the effect was better than that of the polysaccharide medium-dose group (Figure 8); in the high-dose group (200 mg/kg), the liver lobules were relatively intact, and the degree of hepatocyte necrosis was significantly reduced, with a few hepatocytes showing mild edema (Figure 9). Light microscopy results indicated that Luteolin significantly alleviated the liver damage caused by the CCl₄ and olive oil suspensions, and had a significant protective effect on the degeneration and necrosis of hepatocytes.

5. Conclusion

This paper focuses on the extraction of flavonoids from *Artemisia integrifolia* and investigates their activity. The results show that the concentration of flavonoids inhibits the proliferation of lung cancer cells H1299, demonstrating anti-cancer activity. This has guiding significance for the development of natural anti-cancer drugs. However, the mechanism of action requires further refinement and research. The next step is to explore its anti-cancer mechanisms and conduct experiments with flavonoids from *Artemisia integrifolia* in live mice, with the expectation of achieving good results. The content of this paper provides guidance for the development and rational utilization of *Artemisia*

integrifolia and offers theoretical basis for exploring new, green, and health-promoting natural medicines.

Acknowledgments

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