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Studies on the Antitumor Activity of *Duchesnea Indicae* Herba

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Abstract□As a part of finding the biologically active components from *Duchesnea indica* (Andr.)Focke (Rosaceae), antitumor activities of its water soluble fractions have been studied. The fractions were examined for antitumor activity against sarcoma 180 implanted in mice. The antitumor inhibition ratios of the water soluble fractions from *Duchesnea indica* were 17.9, 37.1, 62.7, 60.1, 62.4%, respectively.

Keywords□*Duchesnea indica*, ICR-mouse, Antitumor Activity, Sarcoma 180

The whole plant of *Duchesnea indica* (Andr). Focke (Rosaceae) has been used as a folk medicine in treating amenorrhea, inflammation, fever and traumatic injuries, etc.. A decoction of the plant is taken to treat rheumatism, to cure couth, or as a tonic for tubercular or generally weak people.[1] The juice may be taken to treat fever, as an antidote for arrow poison, also as an antiseptic.[2]

The extensive research for the investigation of biologically active substances from this plant was started only recently. In the chemical researches, Mitsuhashi *et al.* reported the isolation of linolic acid and ß-sitosterol.[3-4] The lupeol, friedelin and ß-amyrin etc. were identified by Mukherjee *et al.* in 1983.[5]

Our laboratory has been reporting on the estrogenic and histaminergic activity from the ether soluble fraction of *Duchesnea indica.*[6] Lee also reported the isolation of sterol compound and the effects on the growth of bacteria from the ether soluble fraction of *Duchesnea indica.*[7-8]

Although the old books have been reported on the antitumor activity of *Duchesnea indica*, the components with antitumor activity from this plant were not found yet.

For this reason, authors studied antitumor activity *in vivo* test from the water soluble fraction of *Duchesnea indica.*

The object of this paper is to find new antitumor activity substances from *Duchesnea indica*, moreover to isolate and identify those biologically active constituents.

In the present study, water soluble fractions extracted from the whole plant of *Duchesnea indica*, were subjected to antitumor test against sarcoma 180 cells in ICR-mice.

Based on the experimental results, it was concluded that the antitumor activity from the water soluble fractions of *Duchesnea indica* was found.

EXPERIMENTAL METHODS

Duchesnea indicae Herba used in this study was collected in Kwang Nung, Kyung Ki province and the suburbs of Seoul during July, 1984.

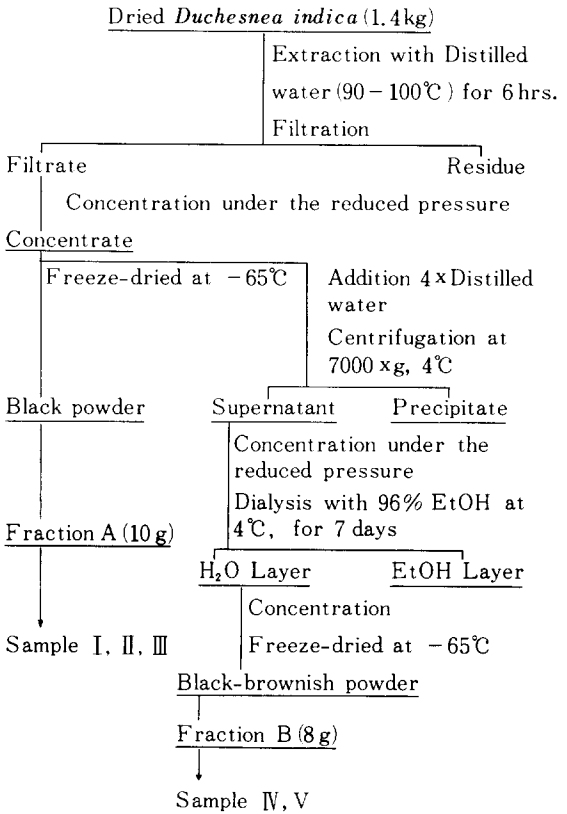
The whole plant of *Duchesnea indica* was used in this study.

Extraction and Isolation

1.4kg of the dried *Duchesnea indica* were cut in small size. Extraction with 1400 ml of distilled water was performed by refluxing on a hot water bath at 90-100°C for 6 hours. These extraction methods were repeated three times. All the filtrates were combined and then concentrated in a rotary vacuum evaporator.

A half of the concentrate was freeze-dried at -65°C. The black powder yielding 10 grams were obtained. (Fraction A)

Another half of the concentrate was mixed with four volumes of distilled water. The supernatant was collected by centrifugation for 30 minutes at 7000 $\times$ g, 4°C (Sorvall RC-5B Refrigerated Superspeed Centrifuge) and concentrated under the reduced pressure. The concentrate was then dissolved with 96% EtOH into dialysis tubes, and allowed to stand at 4°C overnight for 7 days. After dialysis, the insoluble substance was col-



Scheme 1. Fractionation of *Duchesnea indica*.

lected. It was concentrated in a rotary vacuum evaporator and dried at -65°C in a freeze dryer. 8 grams of the black-brownish powder were obtained. (Fraction B)

These powders were used in the following experiments.

Antitumor Experiment

1) Animals

ICR-mice of male sex weighing 18-22g were used for these experiments. They were supplied from the Experimental Animal Farm of Seoul National University.

2) Transplantation of Tumor cells

Sarcoma 180 cells were implanted into the intraperitoneal cavity of the ICR-mice. After seven days of implantation, the animals were killed and sarcoma 180 cells were collected with a syringe in ice-cold bath. This peritoneal cells were washed several times with ice-cold saline. The ascitic fluid was diluted with saline to adjust the tumor cell concentration to 1×10^7 cells/ml with hemacylometer. The 0.1 ml of this suspension was inoculated into the left axillary region of ICR-mice subcutaneously.

3) Preparation of Test solutions

To prepare a test solution for a dose of 50 mg/kg (SA.I), 100mg of the black powder which was obtained from the *Duchesnea indicae* Herba were dissolved in 10ml of saline. Also 200mg of the powder were dissolved in in 10ml of saline for a dose of 100 mg/kg (SA. II). The preparation methods of the SA. III, IV, V were the same manner as the above.

For control, physiological saline was used. These solutions were autoclaved for 20 minutes and stored in a refrigerator.

4) Animal test

To test the extract for antitumor effect as shown in scheme six groups of 7 mice (ICR-mice of male sex weighing about 18-22g) were respectively inoculated with 0.1ml into the left axillary region of the mouse.

Intraperitoneal administration of the samples was initial 3 days after tumor transplantation and continued for consecutive 10 days once a day.

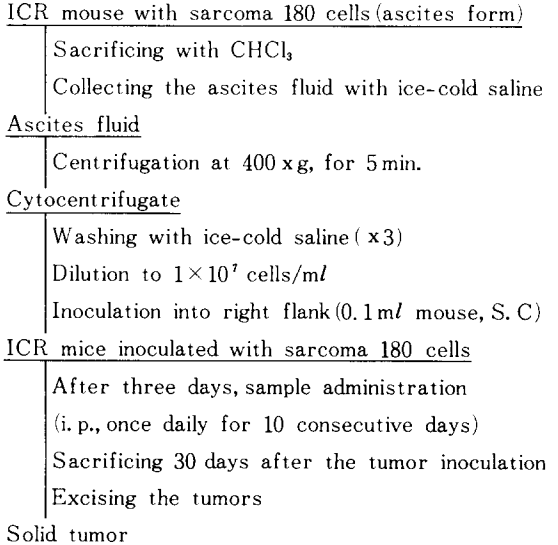
To the first group of 7 mice, 0.1 ml of solution at a dose of 50 mg/kg was injected intraperitoneally. The second group was injected with 0.1ml of the solution at a dose of 100mg/kg. The third group was injected with 0.1ml of solution at a dose of 200mg.

The fourth group was injected with 0.1ml of the solution at a dose of 100 mg/kg of the black-brownish powder. The fifth group was injected with 0.1ml of the solution at a dose of 200 mg/kg of the black-brownish powder.

The last group was injected intraperitoneally with 0.1ml of physiological saline and used as control group.

5) Calculation of inhibition ratio

The tumor weights were measured on the 30th day after implantation and inhibition ratio was determined



Scheme 2. Antitumor test procedure *in vivo*.

by comparision with the tumor weights of control group.

The tumor inhibition ratio was calculated by the following formullus:

$$\text{Tumor inhibition ratio (\%)}: \frac{CW - TW}{CW} \times 100$$

TW: Average tumor weight of each treated group
CW: Average tumor weight of the control group

6) Chemical analysis

The concentration of test solutions containing the extract of *Duchesnea indica* used in the reactions was 1μg/ml in all cases. Anthrone test, Molish test, Iodine test, Xanthoprotein test, Biuret test, and Lowry-Folin test were carried out according to Park[9] *et al.*

7) Total polysaccharide content

Polysaccharide content of the antitumor fraction was determined by the anthrone method using glucose as a standard sugar. The degree of absorption was measured by U.V. spectrophotometer (Shimadzu UV-visible Recording Spectrophotometer UV-240) at 620 nm. Polysaccharide content was calculated from the calibration curve.

8) Total protein content

Protein content of the antitumor fraction was measured by Lowry-Folin method using albumin as a standard protein. The degree of absorption was determined by u.v. spectrophotometer (Shimadzu UV-visible Recording Spectrophotometer UV-240) at 750 nm. Protein content was calculated from the calibration curve.

Table Ⅰ. Antitumor activities of Various Fractions of the *Duchesnea indica*.

Group	Dose mg/kg/day i. p	Average Tumor Wt. (g)	Inhibition Ratio (%)
control	saline	4.58±0.23*	
SA. Ⅰ	50	3.76±0.33	17.9
control	saline	5.80±0.84	
SA. Ⅱ	100	3.65±0.22	37.1
SA. Ⅲ	200	2.16±0.52	62.7
SA. Ⅳ	100	2.31±0.57	60.1
SA. Ⅴ	200	2.18±0.41	62.4

*Mean ± Standard deviation

RESULTS AND DISCUSSION

Antitumor effects of the extract of *Duchesnea indica* on sarcoma 180 in mice were shown in Table I.

In the antitumor effects on sarcoma 180 in mice, the results showed that the water soluble fraction of *Duchesnea indica* had a relatively high antitumor activity in the animal test.

Table Ⅱ. Results of color reactions on the extract from *Duchesnea indica*.

Test	Color	Fraction A	Fraction B
Anthrone test	dark-green	++	++
Molish test	Red-purple	++	+
Iodine test	Brown	+	+
Xanthoprotein test	yellow	+	+
Biuret test	purple-blue	+	++
Lowry-Folin test	dark-blue	++	++

That is, the fraction showed 17.9% tumor inhibition ratio against sarcoma 180 implanted in mice at a dose of 50 mg/kg/day (SA.I).

And 37.1% tumor inhibition ratio was obserbed at a dose of 100 mg/kg/day (SA. II).

Of the 6 fractions tested, SA. III, IV, V showed the tumor inhibition ratio 62.7, 60.1, 62.4% respectively.

The results of color reaction were summerized in Table II. And the chemical analysis of the antitumor fraction showed that it contained a polysaccharide (Fraction A : 2.64%, Fraction B: 22.5%) (Table III).

The results of Lowry-Folin test showed that the protein contents of the extracts were 12.03% (Fraction A) and 4.65% (Fraction B) (Table IV).

The observations obtained in the present study suggest that the polysaccharide and protein fractions of *Duchesnea indica* may be connected with a relatively high antitumor activity. Therefore it is necessary to determine whether polysaccharide and protein fractions of *Duchesnea indica* act as antitumor active substances. Thus we are going to attempt to determine the effects of polysaccharide and protein fractions of *Duchesnea indica* against tumor cell.

And the antitumor active constituents of *Duchesnea in-*

Table Ⅲ. Polysaccharide contents of the antitumor fraction of *Duchesnea indica*.

Sample	Fraction A	Fraction B
Polysaccharide content (%) (after Anthrone test at 620nm)	2.64	22.5

Table Ⅳ. Protein contents in the antitumor fraction of *Duchesnea indica*.

Sample	Fraction A	Fraction B
Protein content (%) (after Lowry-Folin test at 750nm)	12.03	4.65

dica were not found yet. Of course the structures of antitumor active components from *Duchesnea indica* were not found.

Authors are going to isolate and identify the components which antitumor activity of *Duchesnea indicae* Herba.

Moreover, authors expect that we will be able to elucidate their chemical structures by the instrumental analysis and chemical tests.

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