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Antifertility Activity of *Dictamnus albus* Root Bark

Eun Bang Lee, Won Sick Woo, Sam Sik Kang,
Kuk Hyun Shin and Hyung-Joon Chi

Natural Products Research Institute, Seoul National University, Seoul 110, Korea

Abstract—The preliminary antifertility test of *Dictamnus albus*(Rutaceae) showed that a methanol extract of root bark decreased fertility in rats when administered orally on days 1~10 postcoitum. Hexane, chloroform, butanol and aqueous fractions from the methanol extract, were tested to produce the result that only the hexane fraction exhibited positive activity. The hexane fraction was subfractionated into acidic, alkaloidal and neutral subfractions and tested. Neutral subfraction showed positive activity. From the neutral subfraction, fraxinellone was isolated as an active principle. The timed-dosing experiments revealed that antifertility activity of fraxinellone appeared to be exerted after tubal exit of the ova to prevent implantations.

Keywords—*Dictamnus albus* · Rutaceae · fraxinellone · antifertility · antiimplantation

Dictamnus albus (Rutaceae) is described to distribute in Europe and Asia. Ethnomedically this plant has been used to promote monthly flow[1] in Europe, and been used as an emmenagogue in Chile.[2] The hot water extracts have been used for the treatment of amenorrhea[3].

Previous chemical studies have shown that the plant contained the limonoids, limonin[4,5] as well as a lactone fraxinellone.[4,6~8] The furoquinoline alkaloids such as dictamine, skimmianine and γ-fagarine have been also isolated from this plant[4,7,9].

Therefore, the plant has been assayed for antifertility activity in rats. Bioassays for postcoital antifertility activity were performed as following. Sprague-Dawley rats were inbred and fed with rodent chow supplied by Samyang Co. Ltd. and water *ad libitum*. Animals were housed in environmentally controlled room that provided 14 hr of light and 10 hr of darkness for 24-hr period and a temperature of 20±2°. The female rats of 8 weeks of ages were smeared every morning and selected those of proestrus or estrus days of cycle. Male rats of proven breeder were placed into females's cages at around 18:00 hr. The first morning on which evidence of a positive mating was found was designated day 1 of pregnancy. Following mating, females were alternately assigned to the vehicle or one of the treatment groups. Dosing was performed orally from day 1 to day 10 of pregnancy, as otherwise not stated. The test substances insoluble in water were made as their polyvinylpyrrolidone (PVP) co-precipitates to solubilize, as described by Simonelli *et al.*[10] At autopsy on day 16, the number of pregnant animals which had implantation site(s) within each vehicle and treatment group was determined. For each pregnant animals, the number of implantation sites, normal fetuses, degenerating fetuses and corpora lutea of pregnancy were counted.

The methanol extract of the root bark of the plant was found to have antifertility activity at a dose of 2.2 g/kg p.o., as given from day 1 to

day 10 for pregnancy. However, the water and hot water extract did not show the activity. The activity of the methanol extract was retested with newly made extract resulted in positive activity at the same dose level. In order to pursue the compound(s) responsible for the activity, methanol extract was fractionated with hexane, chloroform and butanol and the fractions were tested. The results are shown in Table I.

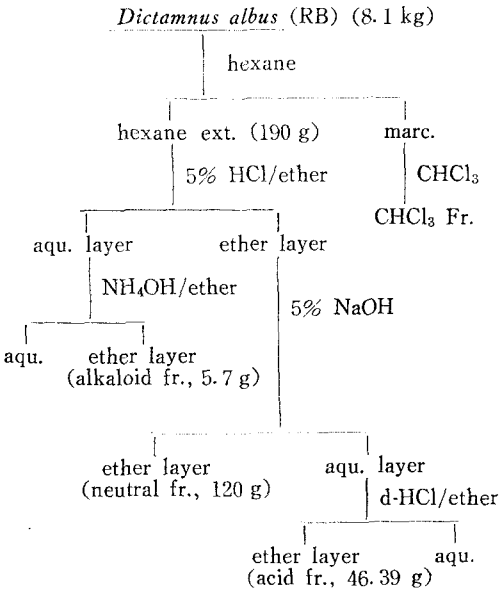
Table I. Antifertility activity of *D. albus* root bark extracts and fractions

Assay No.	Substance[a]	Dose (mg/kg, p.o.)	No. of pregn./ No. of treat.[b]
I	Vehicle (10% PVP)	—	10/10
	MeOH ext.(PVP, 1 : 4)	1.5	7/10
		2.2	6/10[c]
II	Vehicle (10% PVP)	—	10/10
	MeOH ext.(PVP, 1 : 4)	2.2	3/10[c]
III	Vehicle(10% PVP)	—	9/10
	Hexane fr.(PVP, 1 : 4)	1.16	4/8(2)[c]
	$CHCl_3$ fr.(PVP, 1 : 4)	1.37	8/10
	BuOH fr.	1.9	9/10
	H_2O fr.	4.0	9/10

a) The substances were administered, either suspended in 10% PVP soln. or as PVP co-precipitates (1pt. substance: 4 pts. PVP)
b) The figures in parentheses indicate the number of animals died before day 16.
c) $p<0.05$ compared with corresponding vehicle group in χ^2-test.

The hexane fraction has antifertility activity at a dose of 1.6 g/kg p.o., *i.e.* only four animals were pregnant among eight animals. However, chloroform, butanol and water fractions did not show the activity. From the results, it is obvious that the hexane fraction has active principle(s). Therefore, the hexane extract was fractionated into acidic, alkaloidal and neutral fractions, as shown in Scheme 1.

Alkaloidal and acidic fractions at the each dose of 0.07 g/kg p.o. and 0.56 g/kg p.o., showed no activity, whereas neutral fraction at a dose of 1.46 g/kg p.o. was so toxic that all animals



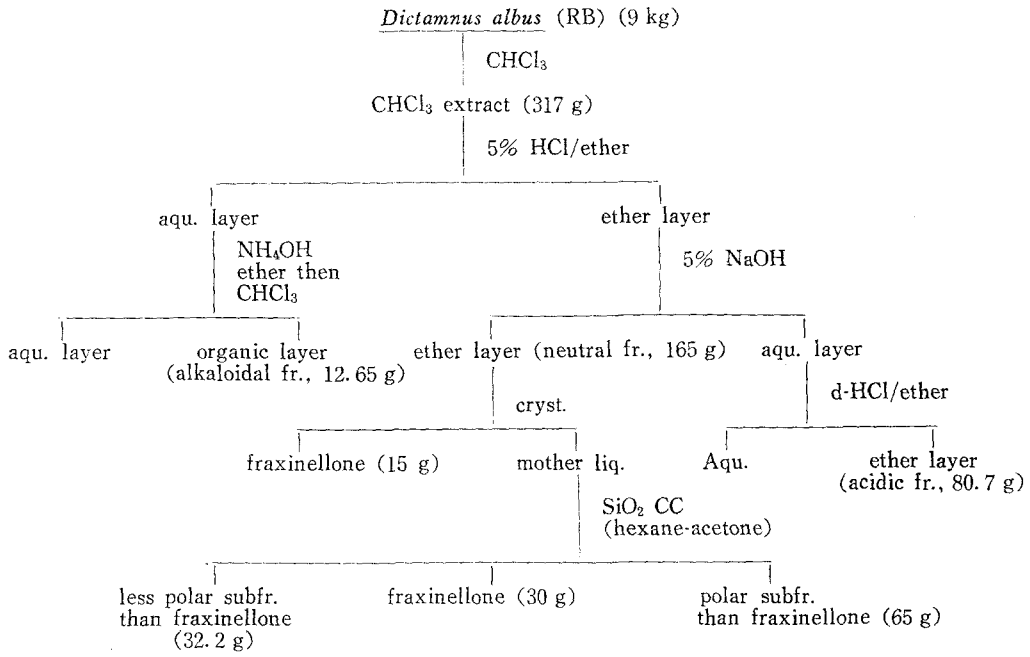
Scheme 1. Fraction of hexane extract.

died (Table II). The doses used were equivalent to 2.3 g of hexane extract. The test at a reduced dose of 0.73 g/kg of neutral fraction showed positive activity with five death out of ten treated animals. This result suggests that active principle(s) might be neutral compound(s). Therefore, the neutral fraction was separated into 3 parts: fraxinellone (major constituent), less polar subfraction than fraxinellone and polar subfraction than fraxinellone (Scheme 2). The

Table II. Antifertility activity of subfractions from hexane extract

Substance[a]	Dose (mg/kg, po)	No. of pregn./ No. of treated.
Vehicle (10% PVP)	—	10/10
Alkaloidal subfr. (PVP, 1 : 4)	0.07	9/10
Acidic subfr. (PVP, 1 : 4)	0.56	10/10
Neutral subfr. (PVP, 1 : 4)	1.46	0/0(6)
	0.73	3/5(5)[b]

a) The substances were administered, either suspended in 10% PVP soln. or as PVP co-precipitates (1 pt. substance: 4 pts. PVP)
b) $p<0.05$ as compared with vehicle group in χ^2-test.
c) The figures in parentheses indicates the number of animals died before day 16.



Scheme 2. Fraction of chloroform extract.

result of bioassay showed that less polar subfraction and more polar subfraction was inactive at the doses of 0.15 and 0.30 g/kg, respectively. Double doses of the subfractions revealed also negative activity as shown in Table III.

However, as shown in Table IV, fraxinellone exhibited positive activity at a dose of 0.075 g/kg. At the doses of 0.106 and 0.15 kg/kg it had positive activity with toxicity shown by death of three and six animals, respectively. In parallel with this experiment EE_2-PVP was used as a positive control. From this result the positive activity of fraxinellone was definitely confirmed.

In order to determine whether the antifertility be attributable to antiimplantation activity or early abortifacient activity, the timed-dosing experiment was carried out. The result showed positive activity when given 0.106 kg/kg on day 5~8 as shown in Table V.

This finding suggests that fraxinellone might interrupt implantation at a dose of 0.106 g/kg. It is well known that the implantation stage is considered to be three stages. Stage 1 begins by the evening of day 5 of pregnancy, when the blastocysts are held in position within the uterine

Table III. Antiferty activity of fraxinellone free neutral subfractions from hexane exract of ***D. albus*** (RB)

Substance	Dose (mg/kg, p.o.)	No. of pregn./ No. of treat.	Implant. site of pregn. rat (M±S.D.)	Normal fetus of pregn. rat (M±S.D.)	Corpora lutea of pregn. rat (M±S.D.)
Vehicle (10% PVP)	—	10/10	11.6±2.2	11.1±2.1	12.0±2.2
Less polar subfr. (PVP, 1 : 3)	0.15	10/10	10.6±1.6	9.9±1.4	11.2±1.9
More polar subfr (PVP, 1 : 3)	0.3	10/10	10.5±2.0	9.6±2.0	10.9±1.8
Vehicle (10% PVP)	—	10/10	10.0±1.8	9.3±2.1	10.8±1.6
Less polar subfr. (PVP, 1 : 3)	0.3	10/10	9.9±1.4	9.5±1.3	10.3±1.8
More polar subfr. (PVP, 1 : 3)	0.6	9/10	10.3±0.9	10.2±1.0	11.0±1.2

Table IV. Antifertility activity of fraxinellone

Substance	Dose (g/kg, p.o.)	No. of pregn./ No. of treat.[a]	Implant. site of pregn. rat (M±S.D.)	Normal fetus of pregn. rat (M±S.D.)	Corpora lutea of pregn. rat (M±S.D.)
Vehicle (10% PVP)	—	10/10	10.5±1.6	9.9±1.7	10.8±2.0
Fraxinellone (PVP, 1 : 3)	0.075	6/10[b]	9.3±3.9	8.8±3.7	11.8±2.4
	0.106	0/7(3)[b]	—	—	—
	0.150	1/4(6)[b]	11.0±0	0±0	13.0±0
Vehicle (10% PVP)	—	10/10	9.6±1.3	9.1±1.4	10.0±1.7
Fraxinellone (PVP, 1 : 3)	0.106	0/7(3)[b]	—	—	—
EE_2-PVP (1 : 4)	0.06 mg/kg	4/10[b]	7.3±3.8	6.0±5.0	11.0±2.6

[a] Figures in parentheses indicate the number of animals died before day 16.
[b] $p<0.05$ compared with vehicle group in χ^2-test.

Table V. Antifertility activity of fraxinellone

Substance	Dose (mg/kg, p.o.)	Treatment days postcoitum	No. of pregn./ No. of treat.	Implant. site of pregn. rat (M±S.D.)	Normal fetus of pregn. rat (M±S.D.)	Corpora lutea of pregn. rat (M±S.D.)
Vehicle (10% PVP)	—	1~10	10/10	10.8±1.1	10.3±1.2	11.0±0.9
Fraxinellone (PVP, 1 : 3)	0.075	1~4	10/10	11.6±2.0	11.3±1.8	12.2±1.9
	0.106	1~4	7/10	12.3±1.5	11.3±1.4	14.0±2.1
	0.075	5~8	10/10	10.6±1.6	10.1±1.6	11.0±1.8
	0.106	5~8	4/10[a]	8.8±2.4	8.3±2.9	11.3±1.3
	0.075	9~11	10/10	11.0±1.6	9.7±3.8	11.5±1.6
	0.106	9~11	10/10	10.4±1.6	8.3±3.7	10.5±1.6

[a] $p<0.05$ as compared with vehicle group in χ^2-test.

lumen. A time for stage 2 is day 6 during which the implantation chamber are formed and stage 3 lasts from the evening of day 6 to the morning of day 7, during which elongation of the implantation sites initiated in the stage 2 continues with extensive decidualization of the stroma.[11]

The acute toxicity of fraxinellone was determined in mice and rats. As shown in Table VI, by intraperitoneal administration of fraxinellone, the LD_{50} value was measured to be 355 mg/kg for mice and 116 mg/kg and 120 mg/kg for male and female rats, respectively. By oral administration, it was 430 mg/kg, 281 mg/kg and 274 mg/kg for male mice, male and female rats, respectively.

It is known that the induction of delivery occurs through uterotonic activity. Thus the compound was tested for the activity in oestrogenized rat uterus, but it has no activity at a high dose of 1×10^{-3} g/ml. On the contrary it showed non-competitive inhibition against the contraction produced by acetylcholine in the

Table VI. Acute toxicity of fraxinellone

Animal	Sex	Route	LD_{50} (mg/kg)	
Mice	Male	i. p.	355[1]	(298.3~422.5)*
		p. o.	430	(376.9~490.6)
Rats	Male	i. p.	116[2]	
		p. o.	281	
Rats	Female	i. p.	120	
		p. o.	274	

* 95% confidence limits
1) Litchfield-Wilcoxon method
2) Reed-Münch's method

uterine muscle[12]. As the skimmianine appeared in the literature to have an embryotoxic effect[13], its antifertility activity was tested which was resulted in negative activity[12].

In conclusion, the methanol extract of *D. albus* root bark was found to have antifertility activity. Systematic fractionation monitoring by bioassay resulted in isolation of fraxinellone as an active compound. It is also revealed that this compound might interrupt the stage of implantation in rats.

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