

Alfalfa 추출물의 생리활성물질의 분리 및 동정

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ISOLATION AND IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM FRESH
ALFALFA EXTRACTS

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Objectives:

Experiments were conducted to attempt to isolate, purify and identify the bioactive compounds that might be responsible for autotoxicity and / or allelopathy and determine their phytotoxicity on germination and seedling.

Materials and Methods:

Isolation and separation procedures used involved an 80% methanol extract of vegetative stage fresh alfalfa leaves (1kg), treatment with activated charcoal, microcrystalline cellulose thin layer chromatography (TLC), followed by Sephadex LH-20 column chromatography. The various fractions were examined further by high performance liquid chromatography (HPLC). This study was repeated twice.

Results and Discussion:

A stepwise separation of crude extract suggested that crude extract minus fat, wax was the most inhibitory. Preliminary results showed that several phenolic compounds assayed for their phytotoxicity on germination and seedling length of alfalfa, chlorogenic acid was the most inhibitory. Preliminary characterization by HPLC analysis resulted in peaks with retention times to close those of chlorogenic and salicylic acid standards, but several other peaks, including the major ones remain unidentified. Flavonoids, such as quercetin and rutin were not detected in any of the fractions; isoflavonoids previously have been reported to be involved in alfalfa autotoxicity and allelopathy. Determination of phytotoxicity by alfalfa seed germination and seedling growth bioassays and the likelihood that chlorogenic acid exists in relatively small amounts in the alfalfa aqueous extract suggest that this compound may be, at least in part, involved in autotoxicity and allelopathy. As alfalfa stage increased, the percentage of chlorogenic acid in MeOH extracts of tissue decreased. Crude saponin extracted from alfalfa inhibited germination of *Chenopodium* and the response depended on the source of extracts and the extract concentration.

Table 7.1. The effect on alfalfa germination of compounds desorbed from various bands isolated from microcrystalline cellulose TLC plates.

Band Number	Range(cm)	Recovery(g)	Germination(%)
Band 1(Top)	16.5-14.5	0.01	90
Band 2(Second from top)	14.5-12.5	0.01	91
Band 3(Third from top)	12.5-10.5	0.01	88
Band 4(Fourth from top)	10.5-8.5	0.01	87
Band 5(Fifth from top)	8.5-6.5	0.42	0
Band 6(Sixth from top)	6.5-4.5	0.43	0
Band 7(Seventh from top)	4.5-2.5	0.30	14
Band 8(Eighth from top)	2.5-0.5	0.17	27
Band 9(Original spot)	0.5-0.0	0.12	37

Table 7.2. The effect on alfalfa germination of various fractions from Sephadex LH-20 column chromatography.

Fraction Number	Volume(ml)	Recovery(g)	Germination(%)
Fraction 1	300	0.01	94
Fraction 2	100	0.02	78
Fraction 3	100	0.14	48
Fraction 4	100	0.31	19
Fraction 5	100	0.42	0
Fraction 6	100	0.28	43
Fraction 7	100	0.02	76
Fraction 8	100	0.01	94
Fraction 9	100	0.01	93
Fraction 10	200	0.01	94

Table 7.6. Germination percentage and seedling length and weight of alfalfa in response to different chlorogenic acid concentrations.

Concentration(M)	GP ¹ (%)	RL ¹ (Cm)	SL ¹ (Cm)	DRLW ¹ (mg)	DRRW ¹ (mg)	DRSW ¹ (mg)
Control	88.6	4.3	3.7	0.72	0.32	0.78
10 ⁻⁴	71.7	3.6	2.5	0.65	0.25	0.67
10 ⁻³	54.6	3.0	3.2	0.53	0.17	0.56
LSD(0.05)	6.1	0.4	0.5	0.07	0.02	0.08
CV(%)	6.2	8.5	11.7	9.1	8.8	8.8

¹ GP, Germination Percentage; RL, Root Length; SL, Shoot Length; DRLW, Dry Leaf Weight; DRRW, Dry Root Weight; DRSW, Dry Stem Weight.

Table 7.5. Germination percentage of *Chenopodium* as affected by leaves and root crude saponin.

Concentration (%)	Germination Percentage (%)	
	Leaves	Root
0	87.5	87.5
2	74.5	62.0
4	66.8	42.3
6	48.8	35.3
8	41.3	23.0
10	25.0	7.3
LSD (0.05)	6.4	4.4

Table 4.3. Effect of various fractions obtained from aqueous extracts of fresh alfalfa leaves on alfalfa.

Fraction Number	Concentration (%)	Germination Percentage	RL* (cm)	SL* (cm)
1	3	100.0	6.0	8.1
	6	73.3	4.4	6.4
	12	58.0	2.6	3.0
2	3	100.0	9.0	9.8
	6	100.0	8.1	8.0
	12	86.1	7.2	9.2
3	3	88.0	5.1	8.4
	6	68.7	4.4	6.3
	12	40.0	2.3	3.0
4	3	84.0	6.4	8.3
	6	85.7	5.4	7.2
	12	86.6	3.9	6.1
5	3	87.0	7.2	8.5
	6	84.6	5.1	7.2
	12	86.6	3.0	4.0
6	3	100.0	7.2	8.6
	6	82.3	5.1	6.7
	12	70.3	3.2	4.4
7	3	100.0	9.4	8.6
	6	89.0	7.6	8.1
	12	100.0	5.2	7.9
8	3	86.6	7.8	8.6
	6	83.3	6.3	7.7
	12	75.3	3.9	5.9
9	3	100.0	8.4	8.7
	6	100.0	7.3	8.0
	12	87.7	6.1	7.8
LSD (0.05)		12.01	0.1	0.1

*. RL = Root Length. **. SL = Shoot Length.

Table 7.7. Quantity of methanol extract, chlorogenic acid obtained from partitioning and HPLC quantification of 100 grams of fresh alfalfa of various stages.

Stage (days)	Material Recovered	
	MeOH Extract (g/100g)	Chlorogenic acid (g/100g) (%)
30	7.5	3.8
60	5.7	1.7
90	3.1	0.9