# Toxicity of Saponins in Alfombrilla (*Drymaria arenarioides*)

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**Highlight: Alfombrilla (*Drymaria arenarioides* H.B.K.) is a highly toxic short-lived perennial of the Caryophyllaceae family found in Mexico. The species has gradually spread northward through Chihuahua and Sonora and now threatens to invade the southwestern United States. Alfombrilla was analyzed for seven common poisonous compounds. Of these, only saponins, which assayed 3.0% of the plant dry weight, were present at toxic levels. Sheep were killed when fed dried alfombrilla at 0.5% of body weight and with saponin extracted from an equivalent weight of plant. When 1-week-old chicks were fed alfombrilla at 2 to 3% of body weight and with an equivalent weight of pure saponin extracted from the plant, they were acutely poisoned. Thin-layer chromatography showed that six saponins were present in alfombrilla.**

Alfombrilla (*Drymaria arenarioides* H.B.K.) is a short-lived perennial of the Caryophyllaceae family and is native to the Mexican states of Chihuahua, Sonora, Durango, Zacatecas, and San Luis Potosi. The plants form dense clumps of vegetation to 45 cm in diameter and 25 cm high. Alfombrilla is highly toxic to cattle, sheep, and goats (Dollahite 1959). Animals show toxic signs when fed the plant (dry weight) at doses of 0.1% of body weight (Jacoby and Morton 1974). Alfombrilla is uniformly lethal when fed at 0.5% of body weight. Losses of cattle from alfombrilla poisoning have been severe in Chihuahua (Sperry et al. 1964; Sperry and Walker 1957). Presently, alfombrilla is not found in the United States, but it is reported within 1 km of the border near Antelope Wells, New Mexico, and south of the Arizona border near Nogales, Sonora, Mexico. Alfombrilla is closely related to inkweed or drymary (*Drymaria pachyphylla* Woot. & Standl.), which is also poisonous and is found from western Texas to southeastern Arizona and southward into Mexico (Lantow 1929; Little 1937; Mathews 1933).

Alfombrilla contains alkaloids, oxalates, and saponins in varying concentrations, but none has been identified as the principal poison. Saponins, however, are the principal poison in other Caryophyllaceae family members that are problems in the United States and Canada. Corn cockle (*Agrostemma githago* L.), bouncing bet (*Saponaria officinalis* L.), and cow cockle (*Saponaria vaccaria* L.) contain toxic concentrations of saponins and are known to poison poultry and livestock (Kingsbury 1964).

The present investigation is part of a cooperative research project between Mexico and the United States. This paper reports the identification of the toxic compound in alfombrilla.

## Materials and Methods

Alfombrilla was collected in vegetative growth in April 1976 at Rancho Experimental La Campana, Chihuahua, Mexico. The plant was dried and sent first to the Animal and Plant Health Inspection Service for treatment to destroy viable seed, and then sent to the Poisonous Plant Research Laboratory, Logan, Utah, for analysis and toxicology studies. The plant was ground to 20 mesh for feeding studies and 40 mesh for chemical analyses.

### Toxicity of Alfombrilla

One sheep and two 1-week-old chicks per treatment were used as test animals. Dried alfombrilla was mixed with water and fed to sheep via a stomach pump at 0.5% of body weight. Alfombrilla was extracted with water on a Soxhlet extractor for 24 hours. One sheep was fed the water extract at a dose equivalent to 0.5% of body weight of dried alfombrilla. A second sheep was fed the residue after water extraction at 0.5% of body weight.

For 7 days 1-week-old chicks were fed dried alfombrilla in No. 4 gelatin capsules at 0.5 and 1.0% of body weight. Alfombrilla was extracted for 24 hours on a Soxhlet extractor. The final volume of the water extract was adjusted so that 1 ml of extract equalled 1 g of plant. The chicks were held overnight without food or water; then the extract was placed inside the crop via a rubber catheter. Chicks were treated with doses that equalled 1, 2, 3, and 4 g of plant. The plant residue was fed daily at 1.0% of body weight for 7 days.

### Tests for Toxic Compounds in Alfombrilla

Alfombrilla was tested quantitatively for tannic acid, soluble oxalates, alkaloids, nitrates, nitro compounds, and saponins, and qualitatively for cyanogenetic glycosides.

### Animal Tests with Oxalates and Saponins

Preliminary tests showed that soluble oxalates and saponins were present in alfombrilla. Soluble oxalates assayed 2% in the material used in the feeding trials. An alfombrilla sample collected in August 1976 contained 4% oxalates. The 1-week-old chicks were given oxalic

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acid dissolved in water at 800 mg/kg. This equalled oxalate content of alfombrilla (at 4% oxalate) fed at 2% of body weight. One sheep as given oxalic acid dissolved in water at 200 mg/kg. This amount equalled the oxalate content of alfombrilla fed at 0.5% of body weight.

Saponins were extracted from 400 g of alfombrilla by the method of Pedersen et al. (1966), which is used to extract large quantities of crude saponins from plants. All extracts and residues were fed to sheep and chicks at doses equalling untreated dried alfombrilla fed at 0.5 and 3.0% of body weight, respectively.

Alfombrilla as quantitatively analyzed for saponins according to the methods of Wang (1969), which is used to extract small amounts of saponins from plants. Pure saponin is obtained by chromatographing the crude saponin on filter paper in a mixture of 1-butanol, 1 M ammonium hydroxide, and 95% ethanol (60:30.5:13, V/V). After development, the fluorescent saponin bands are cut into small pieces, and the saponins are eluted from the paper with 60% methanol, then dried and weighed. Pure saponin obtained from the quantitative analysis was fed to 1-week-old chicks at 0.6, 0.9, and 1.5 g/kg of body weight.

### Chromatography of Saponins

Crude saponin powder extracted from alfombrilla was dissolved in 70% ethanol. A total of 20 mg of crude saponin was chromatographed on thin-layer chromatography (TLC) plates in 1-butanol:acetic acid: water (4:1:2) for 5.5 hours. The plates were dried and then sprayed with a solution of antimony trichloride (3 g of $SbCl_3$, 10 ml of chloroform, 3 mg of vanillin). The plates were heated for 1 min at 118°C and then examined under long-wave ultraviolet light for saponins.

## Results and Discussion

### Toxicity of Alfombrilla

The sheep fed alfombrilla at 0.5% of body weight exhibited general weakness and trembling 3 hours after dosing. The animal became comatose 30 min later and died shortly thereafter. The sheep fed the water extract of alfombrilla became depressed within 4 hours after dosing but then seemed to recover; however, it died during the night, about 60 hours after dosing. The sheep fed the residue from the water extraction at 0.5% of body weight showed no toxic signs.

Chicks fed alfombrilla at 0.5% of body weight became depressed and showed general weakness 4 to 6 hours after treatment on the 2nd day. The chicks became progressively weaker each day and died on the 5th and 7th days after dosing with total doses of 2.5 and 3.5% of body weight, respectively. Both chicks fed alfombrilla at 1.0% of body weight became depressed and showed general body weakness 6 hours after they were fed the first dose. One chick died approximately 44 hours after the first dose was administered (the total of two doses equalled 2% of body weight). The second chick died approximately 70 hours after the first dose (the total of three doses equalled 3% of body weight).

One ml of water extract caused general weakness and depression but the chicks usually recovered within 24 hours. Approximately 50% of the chicks fed with 2 ml of extract died within 48 hours of dosing. The 3-ml dose of alfombrilla extract (equal to 3 g of dried plant) killed all 1-week-old chicks within 6 hours. The 4-ml dose killed all chicks in 3 to 4 hours. Plant residue from the Soxhlet extraction produced no toxic signs in chicks when fed at 1% of body weight for 7 days.

### Tests for Toxic Compounds in Alfombrilla

Alfombrilla tested negative for cyanogenetic glycosides, tannins, and nitro compounds. Alkaloids and nitrates were present in trace concentrations only (0.05% alkaloids, 0.02% nitrate nitrogen). Soluble oxalates assayed 2% in the April sample and 4% in the August sample. Saponins in the April sample assayed by the method of Pedersen et al. (1966) yielded 4.25% crude saponin. Plant material assayed by the method of Wang (1969) yielded 3% saponins. Therefore, the crude saponins were approximately 70% pure.

### Animal Tests with Oxalates and Saponins

Oxalic acid fed to chicks and sheep in amounts equal to that contained in alfombrilla produced no toxic signs. Therefore, soluble oxalates were eliminated as the principal toxic compound in alfombrilla.

Chicks and sheep were unaffected when fed plant residue after extraction with 70% ethanol (I) and residues obtained after extractions with benzene (II) and ether (III) (Fig. 1). The water

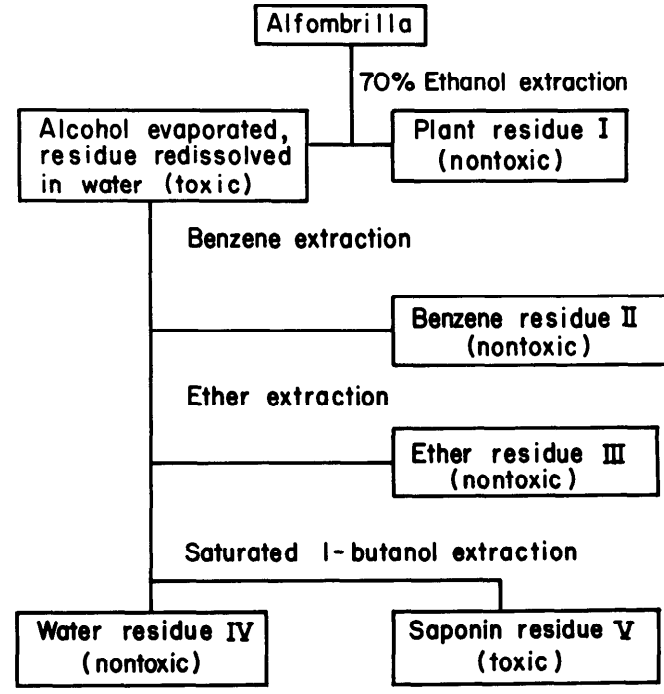


**Fig. 1.** *Extraction of crude saponins from alfombrilla.*

fraction (IV) was nontoxic after 12 extractions with saturated 1-butanol. Preliminary studies had indicated that only 65 to 70% of the saponin was removed from the water phase after six extractions with 1-butanol. Even after 12 extractions, saponins were detected in the water phase by TLC.

The sheep dosed with crude saponin (V) recovered from the saturated 1-butanol extraction appeared normal 4 hours after dosing; however, 5 hours after dosing, the sheep was depressed and stood with its head lowered. Thirty minutes later, the animal was comatose with its head pulled to one side. Breathing was labored and audible. The sheep died before midnight, about 16 hours after dosing.

Chicks fed crude saponin at 2% of body weight were affected 4 hours after dosing and died during the night. All chicks fed pure saponin obtained from quantitative analysis died. All chicks showed depression and stood with heads lowered and eyes closed within 2 hours after treatment. The chicks dosed with 0.6 g/kg of body weight died between 36 and 48 hours after treatment; those dosed with 0.9 g/kg died between 18 to 22 hours; and those dosed with 1.5 g/kg died about 5 hours after treatment. The 0.6, 0.9, and 1.5 g/kg doses represented the amount of saponin from 2, 3, and 5 g of plant, respectively.

All extracts derived from alfombrilla containing saponin fractions were lethal to sheep when fed in an amount equal to 0.5% of body weight of the dried plant. All fractions that did not

contain saponins were nontoxic. The 0.5% amount equalled 150 mg of saponin/kg of body weight.

The $LD_{50}$ for a 100-g chick given a water extract of alfombrilla was 2% of body weight; $LD_{100}$ was 3% of body weight. A water extract dose as low as 1% of body weight occasionally produced acute poisoning but usually produced only depression and weakness from which the chicks eventually recovered. A water extract dose that represented 1% of body weight as dried plant equalled a dose of 300 mg of saponin/kg.

In both sheep and chicks, the lethal dose of saponin corresponded to an equal concentration of saponin found in a lethal dose of dried alfombrilla. Neither sheep nor chicks died as rapidly from extracted saponin as from untreated plant. Saponins are not readily absorbed into the circulatory system. Saponins are thought to possess an irritant, or some other such compound must be present in the plant to injure the wall of the digestive tract and thereby permit absorption of saponin (Kingsbury 1964). Possibly, at least part of the irritant required to rupture the wall of the digestive tract was separated from the saponin during extraction. If so, the absence or reduced concentration of the irritant would explain the additional time required for death of sheep and chicks acutely poisoned by purified alfombrilla saponins.

### Chromatography of Saponins

Six saponins were chromatographed from alfombrilla (Table 1). Bands 1, 3, and 5 fluoresced blue under long-wave ultraviolet light; bands 2, 4, and 6 fluoresced yellow. The magnitude of the fluorescence and the width of the bands were greatest in bands 1, 2, and 3. The saponin of band 6 fluoresced faintly in a narrow band so that this saponin appeared to occur in the lowest concentration. No information is presently available as to the toxicity of the individual saponins.

Seed of alfombrilla was not available for analysis. Saponin content of *A. githago* seed was reported to be 5 to 7% (Kingsbury 1964). If such levels of saponin are present in alfombrilla seed, the plants would be more toxic during seed production.

**Table 1. Color and $R_f$ values of saponins in *Drymaria arenarioides* under long-wave ultraviolet light.**

| Saponin band no. | $R_f$ | Color |
|---|---|---|
| 1 | 0.83 | Bright light blue |
| 2 | 0.65 | Bright yellow |
| 3 | 0.59 | Bright light blue |
| 4 | 0.43 | Light yellow |
| 5 | 0.36 | Pale light blue |
| 6 | 0.21 | Pale yellow |

## Literature Cited


**Dollahite, J. W. 1959.** Toxicity of *Drymaria arenarioides* for cattle, sheep, and goats. J. Amer. Vet. Med. Ass. 135:125-127.

**Jacoby, P. W., Jr., and H. L. Morton. 1974.** Alfombrilla. Univ. of Arizona Coop. Ext. Serv. Agr-File Q-352. 2 p.

**Kingsbury, J. M. 1964.** Poisonous Plants of the United States and Canada. Prentice-Hall, Inc., Englewood Cliffs, N.J. 626 p.

**Lantow, J. L. 1929.** The poisoning of livestock by *Drymaria pachyphylla*. New Mexico Agr. Exp. Sta. Bull. 173. 9 p.

**Little, E. L., Jr. 1937.** A study of poisonous drymaria on southern New Mexico range. Ecology 18:416-426.

**Mathews, J. P. 1933.** The toxicity of *Drymaria pachyphylla* for cattle, sheep and goats. J. Amer. Vet. Med. Ass. 83:255-260.

**Pedersen, M. W., D. E. Zimmer, J. O. Anderson, and C. F. McGuire. 1966.** A comparison of saponins from Du Puits, Lahontan, Ranger, and Uinta alfalfas. *In:* Proc. 10th Int. Grassland Cong., Helsinki, Finland. p. 693-698.

**Sperry, O. E., J. W. Dollahite, G. O. Hoffman, and B. J. Camp. 1964.** Texas plants poisonous to livestock. Tex. Agr. Exp. Sta. Bull. B-1028. 59 p.

**Sperry, O. E., and A. H. Walker. 1957.** Alfombrilla causing heavy losses to livestock in Chihuahua, Mexico. The Cattleman 43:36, 60.

**Wang, Li-Chun. 1969.** Quantitative evaluation of saponin content in Du Puits alfalfa folliage (*Medicago sativa* L.) with *Trichoderma*. PhD Diss. Utah State Univ., Logan. 60 p.