Characterization of the Saponin Fraction of *Tribulus terrestris*

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Tribulus terrestris (puncture vine) was introduced from southern Europe and was reported to have a range from Florida to California by Kingsbury (1964). Although this plant is not considered to be a problem with livestock in Oklahoma, it has been shown to produce hepatogenic photosensitivity in range animals. The same species of plant was reported as the major cause of bighead in sheep in South Africa and Australia by Steyn (1934). The review by Kingsbury (1964) states that a number of steroidal saponins have been isolated from *Tribulus* in South Africa. The reference to work by Boughton and Hardy (1935) in Kingsbury's review does not refer to this plant. Original reports of the work conducted in South Africa (Steyn, 1934) are not available. Hershey (1945) surveyed the plant as a range problem in New Mexico and reported symptoms in the natural cases of puncture vine toxicity as "typical lesions of severe photosensitization or bighead, including blindness, necrosis of skin, loss of lips and ears and a high mortality among young animals". Although saponins are present in puncture vine, the toxicity symptoms are not those expected for these compounds. Saponins show a hemolytic effect by forming molecular complexes with cholesterol in erythrocyte membranes. The mode of action of saponins on cell membranes has been studied by Bangham and Horne (1962). The biological role of saponins in plants is not known but some research workers attribute a pesticide function to this type of compound.

Experimental Procedure

The isolation of crude saponin followed the procedure of Shaver, Camp and Dollahite (1964). Fresh or finely ground dried plant material was extracted three times with a 70% ethanol-water solvent. The extracts were combined, concentrated until all alcohol was removed, filtered and acifified by passing through a hydrochloric acid activated I.R.C. —50 ion exchange column. The saponins were extracted into *n*-butanol and highly colored samples were treated with activated carbon. The butanol solutions were filtered and concentrated to a thick syrup. The last traces of water were removed with absolute ethanol and the saponins were precipitated from this solvent in most cases.

Characterization tests on the saponin isolates were adapted from Clark (1964) and included a Molisch test for carbohydrates, two tests for steroidal-type compounds (Salkowski and Lieberman-Burchard), and a precipitation test with cholesterol. A paper chromatographic separation of glycosides present in these preparations was made with two solvent systems. A Sephadex G-15 column was also utilized as a method of further purification of the isolate. The presence of saponin was first detected with carbohydrate test reagents and then verified by its hemolytic effect on washed erythrocytes.

Results

White powders were isolated as products from seed and stem separately. The isolate of the entire plant tissue was a light-brown powder. The yields from both leaf and root were too low to be precipitated from ethanol and were therefore kept in aqueous solution. A comparison of the saponin activity of isolates from various plant parts as well as the yield is shown in Table I. Table II shows the color tests on different isolates as compared with commercial saponin and digitonin.

TABLE I. YIELD, ACTIVITY AND PURITY OF SAPONIN FRACTIONS* OF *Tribulus terrestris*

Plant Tissue	Saponin Yield	Saponin Activity: Concentration	Saponin Activity: RBC Hemolysis**	Purity FeCl,†
	%		%	
Root	< 0.010	Unknown	140	(—)
Leaf	< 0.010	Unknown	100	(—)
Stem	0.101	1 mg/10 ml	0	(—)
Seed	0.013	1 mg/10 ml	0	(+)
Entire Plant	0.053	1 mg/10 ml	130	(+)
Commercial Saponin		1 mg/10 ml	100	(+)

* 70% Ethanol extract, concentrated and purified.

** Red Blood Cell (RBC) hemolysis by commercial saponin (1 mg saponin/10 mg saline + 2 ml washed RBC suspension).

† Test for phenolic impurities (pigments); (+) indicates presence of impurities.

TABLE II. CHARACTERIZATION TESTS ON SAPONIN ISOLATES

Plant Tissue	Salkowski*	Liebermann-Burchard*	Molisch**	Cholesterol Complex†
Root	++	++	+++	–
Leaf	+++	+++	+++	–
Stem	+	+	+	–
Seed	–	–	–	–
Entire Plant	++	+++	+	–
Commercial Saponin	++	+	++	–
Digitonin	+	++	++	+

* Test for sterol nucleus.

** Carbohydrate color test.

† Test for digitonin-type structure. (The results of this test are subject to a concentration factor.)

Results of paper chromatography in two different solvent systems are given in Table III. For the leaf sample, the saponin activity of spots with different R_f were also checked.

The chromatogram from a Sephadex G-15 column did not show a good separation, but other Sephadex types, designed for high molecular weight substances, may be more effective in fractionation.

DISCUSSION AND CONCLUSIONS

Partial characterization of the saponin components of puncture vine indicate that the saponin content varies with plant tissue. The saponin content in stem and seed were undetectable, whereas the leaf and root tissues appear to have very active saponins. The characterization tests indicate the presence of a sterol nucleus and carbohydrate. A check on the hemolytic activity of different spots from paper chromatography on leaf tissue shows that three different saponins were present. Further work in this area must await the development of a more effective purification procedure.

LITERATURE CITED

Bangham, A. D. and R. W. Horne. 1962. Action of saponin on biological cell membranes. Nature 198:952.

Boughton, I. B. and W. T. Hardy. 1935. Mescal bean *(Sophora secundiflora)* poisonous for livestock. Texas Agr. Exp. Sta. Bull. 519.

Clark, J. M., Jr. 1964. *Experimental Biochemistry.* W. H. Freeman and Co., San Francisco, California.

Hershey, A. L. 1945. Some poisonous plant problems of New Mexico. N. Mex. Agr. Exp. Sta. Bull. 322.

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

Shaver, T. N., B. J. Camp and J. W. Dollahite. 1964. The chemistry of a toxic constituent of *Xanthocephalum* species. Ann. N. Y. Acad. Sci. 3:737.

Steyn, D. G. 1934. The toxicology of plants in South Africa. Central News Agency Ltd. Johannesburg.

TABLE III. PAPER CHROMATOGRAPHIC SEPARATION OF SAPONIN FRACTION COMPONENTS

Plant Tissue	Solvent 1*		Solvent 2**	
	Spots	R_f Values	Spots	R_f Values
Root	3	0.0, 0.42, 0.87	2	0.60, 0.87
Leaf	4	0.0†, 0.41, 0.61†, 0.77†	3	0.57, 0.69, 0.82
Stem	2	0.04, 0.09	0	0.0
Seed	2	0.03, 0.08	0	0.0
Entire Plant	3	0.0, 0.06, 0.12, 0.38	1	0.82
Commercial Saponin	2	0.34, 0.53	2	0.19, 0.58

* Solvent 1: isopropyl alcohol — acetic acid — water (3:1:1).

** Solvent 2: pyridine — butanol — water (3:2:1).

(Detection spray: periodate — benzidine; 3 mm Whatman No. 1 paper.)

† Hemolytic activity.