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What to Feed the Rhinoceros: Quantification of Tannins and Phenolic Glycosides in Carolina Willow Leaves.

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Abstract

BACKGROUND: Captive black rhinoceros (*Diceros bicornis*) develop iron storage disease and this can lead to severe liver damage. Thus, dietary iron absorption must be regulated for these herbivores. A potential method of controlling iron absorption is to use compounds that are naturally present in the food to bind and immobilize the iron. Carolina willow (*Salix caroliniana*) is a major component of the diet of herbivores at Disney's Animal Kingdom. Willow contains phenolic glycosides and condensed tannins, both of which have the ability to chelate metals. These chemicals may lower the amount of iron available to tolerable levels. The objective of this research is to develop methods to quantify phenolic glycosides and condensed tannins in leaf samples.

METHODS: Condensed tannins were extracted from *S. caroliniana* leaves for use as standards. Leaf sample solutions were analyzed by a tannin depolymerization reaction (butanol-HCl assay) and compared with purified tannin standard. The quantity of tannins in each sample was determined by spectrometry with absorbance at 550 nm. Quantities of the phenolic glycosides, salicin and tremulacin, were determined via HPLC. Leaf phenolic glycosides were extracted using methanol and analyzed by HPLC on a reversed phase column with a 0.1% TFA/acetonitrile eluent gradient. The quantities of both salicin and tremulacin were determined using standard curves. Fractions were collected and analyzed by ESI(Electrospray Ionization)-MS.

RESULTS: Tannin levels in 19 samples ranged from 0.18 to 0.97% by mass. For phenolic glycoside quantification, it was determined that 0.1% TFA in the mobile phase resulted in baseline separation of tremulacin and salicin. Eighty samples of plant material were analyzed and no salicin and/or tremulacin was detected in any of these samples. Our detection limit is 0.8 ppt. ESI-MS was used to verify the identity of the purified phenolic glycosides.

CONCLUSION: *S. caroliniana* leaves are high in tannins and could potentially chelate iron and thus make the dietary iron less bio-available. They have no detectable phenolic glycosides,

which tend to be bitter tasting and less appealing to herbivores. In sum, *S. caroliniana* has levels of tannins with the potential to help in iron storage disease in captive browsing mammals.

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Keywords

hplc, phenolic glycosides, condensed tannins