

The Chemistry Of Low-Molecular Weight Chromones And Glycoproteins From Aloe vera

By Dr. Akira Yagi

School of Pharmacy and Pharmaceutical Sciences

Fukuyama University, Japan

When examining the chemistry of low molecular weight phenolic and chromone components of the latex exudate and glycoproteins from the gel of Aloe vera, there are three areas to consider.

First, the isolation and structure determination, based on spectroscopy, of three new chromones (compound 1, 2, 3) from the leaves were examined. Absolute configuration of the hydroxy propyl group in these compounds was also established. In compound 1, the hydroxy propyl group was in the (S) configuration while that of compounds 2 and 3 were in the (R) configuration. These compounds were identified to be 8-C-glycosyl-7-O-methyl-(S)-aloesol, isoaloeresin D, and aloeresin E, respectively.

Compounds 2 and 3 exhibited inhibitory activity against mushroom tyrosinase, an indicator of skin whitening activity, while compound 1 did not. This difference perhaps could be attributed to the 2' substitution pattern.

The second area involves a rapid and efficient method based on C-18 reversed phase HPLC using UV detection at 290 nm for the analysis of phenolic components {aloesin, 2'-O-feruloyl aloesin, aloeresin-A, barbaloin, isobarbaloin, aloenin, aloe-emodin and compound 1,2 and 3}, at a detection limit of 0.04~0.35 ng per injection, in aloe species. Samples for HPLC analysis were prepared by multiple extractions of the sample (Aloe or commercial aloin) with methanol followed by centrifugation and filtration. The method was applied successfully to the analysis of the components in Aloe vera, *A. arborescens*, *A. vera* var. *chinensis*, *A. marlothii* and *A. striata* as well as in commercial aloin. The distribution of chromone and anthrone compounds were highly variable amongst these aloe species e.g. aloenin was found only in *A. arborescens*, and aloeresin D and E were present in *A. barbadensis* while levels of aloesin and barbaloin varied greatly throughout all species tested.

Finally, the isolation and identification of the glycoprotein fractions from Aloe vera gel was examined. Fractionation of Aloe vera gel by size exclusion chromatography, after dialysis at 8000 mw to remove the smaller molecular species, on DEAE Sephadex-A-25, sepharose 6 B and sephadex G-50 gave a glycoprotein fraction with an in vitro cell proliferation-promoting activity on human dermal normal fibroblasts and baby hamster kidney-21 cell lines. This material showed only one band on both native and sodium dodecylsulfate (SDS) denaturing PAGE. The molecular weight of the material was 29 kD with a PI of 6.8. Immunoblotting of this material after SDS-PAGE showed the material was composed of two 14 kD subunits. Deglycosylation of the material with trifluoromethanesulfonic acid provided a protein with a molecular weight of 13 kD. Although the neutral polysaccharide fractions obtained from the fractionation of Aloe vera gel showed no activity in the assay, another glycoprotein fraction isolated showed inhibitory activity. Based on SDS-PAGE the molecular weight of this fraction was 15~18 kD. Acid catalyzed hydrolysis of this material using 10 % H₂SO₄ gave phenolic substances. Immunodiffusion analysis was performed on the glycoprotein fractions of *A. vera* using rabbit antibodies to these purified fractions. The glycoprotein fraction with cell proliferation promoting activity showed an immunodiffusion precipitation line against rabbit antiserum (Ouchterlony test) at a concentration of 180 ug/mL. Immunodiffusion analysis also showed a precipitation line against the non dialysate of Aloe vera gel at a concentration of 1.25 mg/mL. No Ouchterlony precipitation line was observed with the non-dialysate of *A. arborescens* at a maximum concentration of 5 mg/mL. This method provides a method to characterize and detect the cell proliferation components in Aloe vera gel.