CHEMICAL STUDIES OF *ALOE VERA* JUICE II

Inorganic Ingredients

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In earlier papers one of us [1], [2] reviewed the present status of *Aloe vera* juice as a cure-all. Whereas, we were not strongly convinced of its panacea-like properties, the numerous claims made for it warranted an analysis of the amino acid content of the juice [2] because such compounds may constitute important medicinals. Since we are aware that another institution is studying its carbohydrate content, we felt that an investigation of the mineral constituents might be of interest. It is well known that some folkloric remedies may, for example, cure cramps if they are caused by Ca deficiency, due to their relatively high content of calcium.

It was felt that an elegant method for the testing of inorganic ingredients of the juice might be neutron activation analysis, and we are greatly indebted to the Nuclear Reactor Laboratory of our Department of Mechanical Engineering for placing their expensive instruments at our disposal, and for the staff's kind cooperation.

The material used in these assays was the same lyophilized extract of *Aloe vera* juice employed in our previous work [2].

Basic Principles of Neutron Activation Analysis [3-6] — Since this method has not been widely employed for the analysis of natural products, the following rather detailed explanation is offered.

Neutron activation analysis is based on the principle that stable isotopes when bombarded with neutrons undergo nuclear transmutations to produce radioactive nuclei. The artificial radioisotope thus produced, may subsequently be identified and assayed quantitatively by determining the energy and the intensity of its gamma emissions.

In the absence of competing reactions, the amount of a given radioisotope created during irradiation, is proportional to the weight of the original stable isotope present in the sample. Additional information useful in the identification of the various elements may be obtained by measuring the characteristic half-lives of the radioactive isotopes produced. Since activation analysis involves nuclear reactions, it determines only the total content of the element sought, not the nature of the chemical bonding in the material.

Equipment Required for Neutron Activation Analysis — The University

of Texas TRIGA nuclear reactor was used for the activation of the *Aloe vera* samples analyzed in this work. The reactor is of the 'swimming pool' type. It is usually operated at a steady-state power level of 250 kilowatts but may be pulsed for approximately 20 milliseconds to a power level of 250,000 kilowatts.

The 'rotary specimen rack', one of several facilities available for irradiation of samples in the TRIGA core, was used for the irradiation. This facility can accommodate a maximum of 40 5" x 1" cylindrical sample containers.

Gamma-ray spectrometry facilities include a thallium activated sodium iodide, NaI(Tl) detector as well as a lithium-drifted germanium, Ge(Li), detector connected to a Nuclear Data (Model 2200) multichannel analyzer system. The Ge(Li) detector was used exclusively for the *Aloe vera* determinations. The relative merits of the two types of detectors will be discussed in the section dealing with the sensitivity of neutron activation analysis.

A simplified diagram of the counting system used is shown in Fig. 1. Gamma-rays which are absorbed in the detector are converted to voltage pulses with amplitudes that are proportional to the energy of the absorbed gamma photons. After amplification, the pulses are sorted by the multichannel analyzer system according to pulse height (*i.e.*, the gamma-ray energy). The analyzer thus accumulates a 'gamma energy spectrum' which consists of the number of gamma emissions detected by the crystal as a function of their energy (Fig. 3).

Sensitivity of Neutron Activation Analysis — A total of 76 of the naturally occurring elements may be detected in quantities of less than 100 μg by neutron activation analysis techniques [6]. Thermal neutron activation in a nuclear reactor may be used to determine 73 elements, the three remaining ones (N, O, and Y), not normally sensitive to thermal neutron activation, may be detected by fast neutron activation in a fast neutron generator or a linear accelerator. The elements which are not readily detected by activation analysis (excluding artificially produced elements and naturally radioactive nuclei) include H, He, Li, Be, B, C, and Tl. Fig. 2 diagrams the elements which may be detected by instrumental activation analysis and gives close approximation of the smallest amount in micrograms of each element (in the absence of interfering activities from other elements) that could be detected with a 20 cm^3 Ge(Li) detector system *. These estimates are based on a neutron flux of 1.8 x 10^{12} n/cm^2-sec. and a one-hour irradiation period. In many cases it is possible to improve significantly the sensitivity by performing simple radiochemical separations. The limits of Fig. 2, however, are based on the assumption that only instrumental (not radiochemical) tech-

* Calculation by Buchanan [7]) of sodium iodide sensitivities were converted using estimated Ge(Li) system efficiencies to the sensitivity limits presented in Fig. 2.

niques are used. It has also been assumed that the lower counting limit is 1000 photopeak cpm for isotopes with half-lifes of less than 1 minute, 100 cpm in the photopeak for those with half-lifes of 1-60 minutes, and 10 cpm for those with half-lifes $\geq$ 1 hour.

The limits of instrumental analysis with the Ge(Li) detector (Fig. 2) are approximately 20 times less sensitive than those calculated by Buchanan [7] for a 3" x 3" NaI(Tl) system. These limits are, however, estimated for an ideal case of no interference from other radionuclides. For analyses that require the determination of several elements in the same sample (as in our case) the superior energy resolution of the Ge(Li) detector provides significant advantages. *The resolution attainable with the latter system allows separation and simple analyses of peaks in spectra that would be difficult to analyze with the more sensitive NaI(Tl) systems.*

Aloe vera Analysis — Samples (approximately 200 mg accurately weighed) of the lyophilized juice were sealed in cylindrical polyethylene vials (1" x $^1/_2$"). Care was taken not to contaminate the sample with foreign materials. Standards of each element under study were treated in an identical manner.

Preliminary tests indicated that the elements that may be identified by examination of the gamma-ray energy spectrum from the *Aloe vera* juice included Na, Cl, Ca, K, and trace quantities of Mn. The standards used were A. R. grade compounds of Na_2CO_3, NH_4Cl, $CaCO_3$, KNO_3, and $MnSO_4$. As indicated in Fig. 2, the CO_3, NH_4, NO_3, and SO_4 ions are not activated in the reactor, consequently, they do not contribute to the gamma-ray spectra of the standards.

The analytical samples were irradiated for three minutes at a thermal neutron flux of $1.8 \times 10^{12} n/cm^2$-sec. The *Aloe vera* and standards were each counted arbitrarily for 400 seconds in the previously described lithium-drifted germanium counting system at arbitrary 'delay times' of 1 minute, 2 hours, and 10 hours.

Typical gamma-ray energy spectra obtained from the *Aloe vera* juice are shown in Fig. 3. Curve No. 1 shows a spectrum determined 1 minute after neutron bombardment. Table 1 identifies each peak from Curve No. 1 and indicates the element with which it is associated. Gamma-rays from the various elements are emitted at a specific energy E, and a 'total absorption' peak (see Fig. 3, Curve No. 1, Peak No. 8 as an example) results at E when the entire energy of the gamma is absorbed in the detector crystal. 'Single escape' and 'double escape' peaks (see Fig. 3, Curve No. 1, Peaks No. 4 and 2, respectively) are also observed at (E-0.51 Mev) and (E-1.02 Mev) as a result of incomplete absorption of the gamma-rays in the detector [3]. Finally, a 'Compton edge' (see Fig. 3, Curve No. 2, Peak No. 8) results because

Table I

Curve No. 1 Identification of Gamma Energy Spectrum Peaks

Peak	Energy	Element	Type of Peak *
1	.51	All	Annihilation **
2	.63	Cl	Double escape
3	.85	Mn	Total absorption
4	1.13	Cl	Double escape and single escape
5	1.37	Na	Total absorption
6	1.45	Cl	Compton edge
7	1.50	K	Total absorption (no distinct peak is observed)
8	1.65	Cl	Total absorption and single escape
9	1.73	Na	Double escape
10	1.93	Cl	Compton edge
11	2.08	Ca	Double escape
12	2.15	Cl	Total absorption
13	2.24	Na	Single escape
14	2.59	Ca	Single escape
15	2.75	Na	Total absorption
16	3.03	Ca	Double escape (from 4.05 Mev Calcium gamma)
17	3.10	Ca	Total absorption

* For further explanation see text.

** An 'Annihilation' peak can be observed as a result of the absorption of gammas by pair production in materials surrounding the detector and the subsequent detection of 0.51 Mev photons.

Table II

Curve No. 2 Identification of Gamma Energy Spectrum Peaks

Peak	Energy	Element	Type of Peak *
1	.51	All	Annihilation **
2	.85	Mn	Total absorption
3	1.16	Na	Compton edge
4	1.37	Na	Total absorption
5	1.50	K	Total absorption
6	1.73	Na	Double escape
7	2.24	Na	Single escape
8	2.51	Na	Compton edge
9	2.75	Na	Total absorption

* For further explanation see text.

** An 'Annihilation' peak can be observed as a result of the absorption of gammas by pair production in materials surrounding the detector and the subsequent detection of 0.51 Mev photons.

gamma radiation is scattered in the detector crystal and again only part of the total gamma energy is deposited in the detector. At the threshold energy for this phenomenon, given by the expression $\frac{E}{1 + mc^2/2E}$, the 'Compton edge' is observed.

Curve No. 2 shows the spectrum obtained from the same sample counted 10 hours later and Table II identifies the peaks of same. It should be noted that the isotopes with short half-lifes (i.e., Cl-38 with $t_{\frac{1}{2}}$ = 37.3 min. and Ca-49 with $t_{\frac{1}{2}}$ = 8.80 min.) have decayed and only the more stable isotopes remain (i.e., Na-24 with $t_{\frac{1}{2}}$ = 15.0 hrs. and K-42 with $t_{\frac{1}{2}}$ = 12.5 hrs.).

The weight of each element present in the sample was calculated by the 'comparator' method. In this method the juice samples and each of the standards were irradiated and counted under identical conditions so that the amount of an element contained in a unknown sample was determined from the expression $W_u = W_s \times \frac{A_u}{A_s}$, where W_s is the weight of the standard (if the standard is a compound, W_s is only the weight of the element of interest), W_u is the weight of element sought in the unknown sample of *Aloe vera* juice, A_s is the area under a 'total absorption' peak obtained from the standard, and A_u is the area under the corresponding 'total absorption' peak obtained from the unknown sample of *Aloe vera* juice.

To check the results and improve the overall accuracy of the determination, the above procedure was repeated three times and the results were averaged. These results are summarized in Table III. The average standard deviation on successive determinations was ± 2.6 %.

Table III

Inorganic Composition of *Aloe Vera* Juice

Element	Wt % Present in *Aloe Vera* Juice
Ca	4.70 ± 0.10
Cl	12.2 ± 0.2
Na	1.45 ± 1.03
K	6.60 ± 0.3
Mn	.0122 ± 0.0003

Summary

Aloe vera juice (lyophilizate) was found, as determined by neutron activation analysis, to contain 4.7 % Ca, 1.43 % Na, 6.6 % K, 12.2 % Cl, and 0.01 % Mn. The concentrations of Cl and K appear, as far as general plant products are concerned, to be higher than expected, whereas the Na

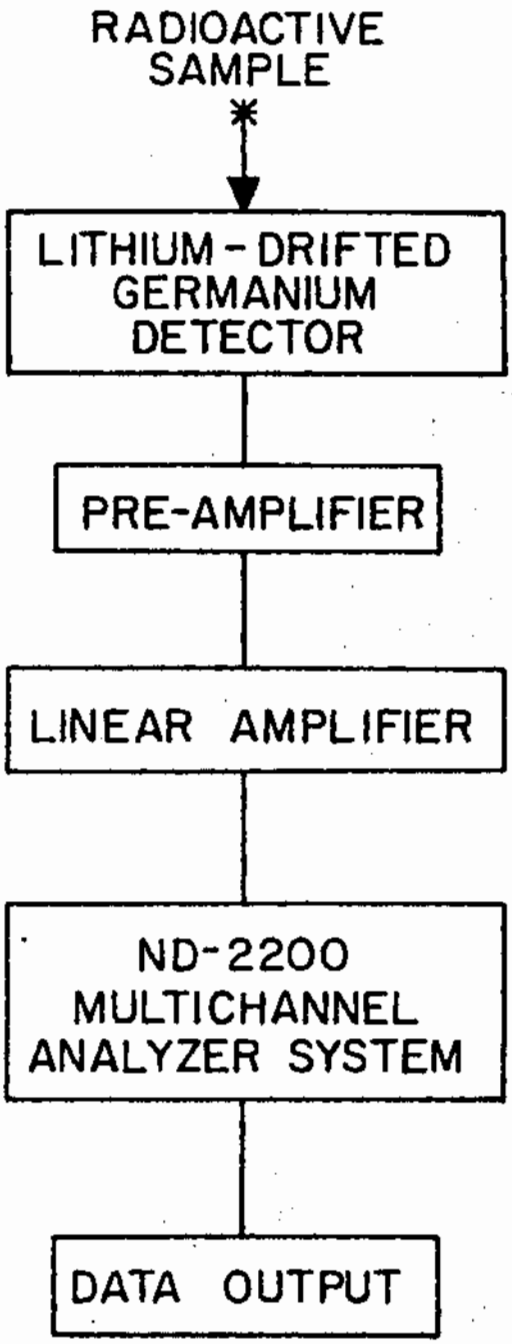
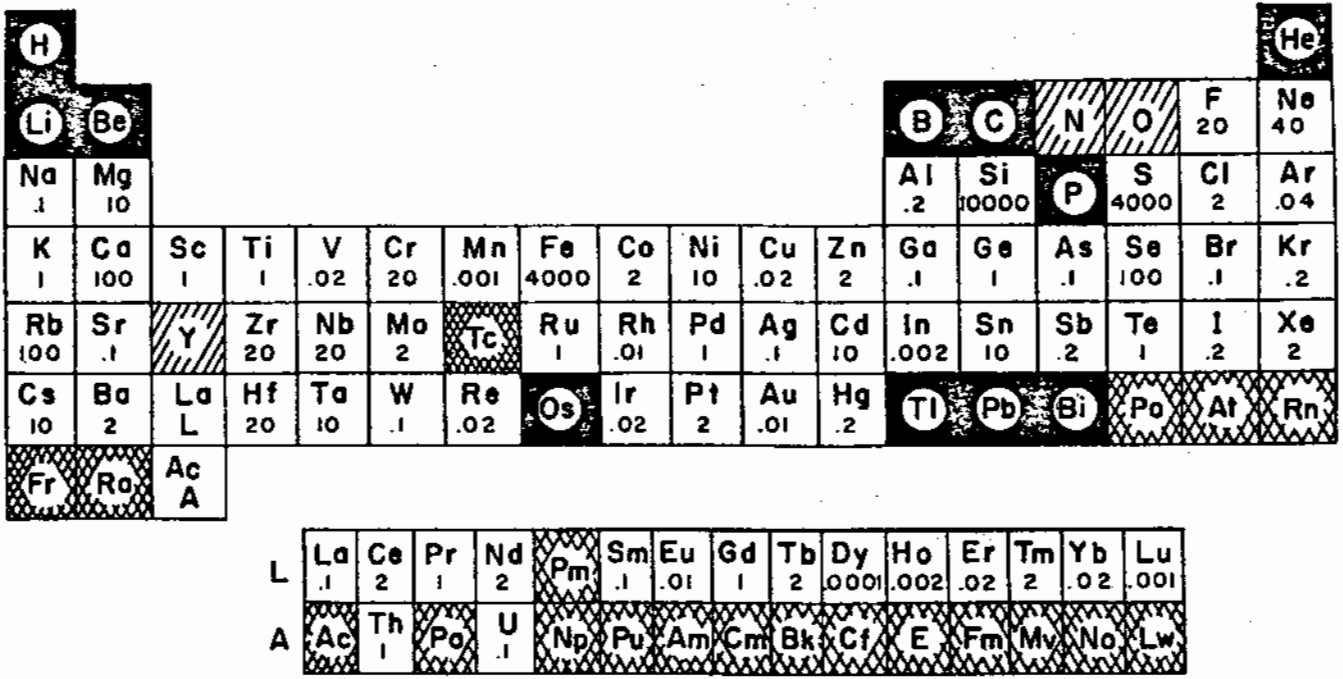


Figure 1 — Schematic Diagram of Lithium-drifted Germanium Counting System.



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☐ Sensitive to instrumental thermal neutron activation analysis (smallest amount that can be detected is given in micrograms).

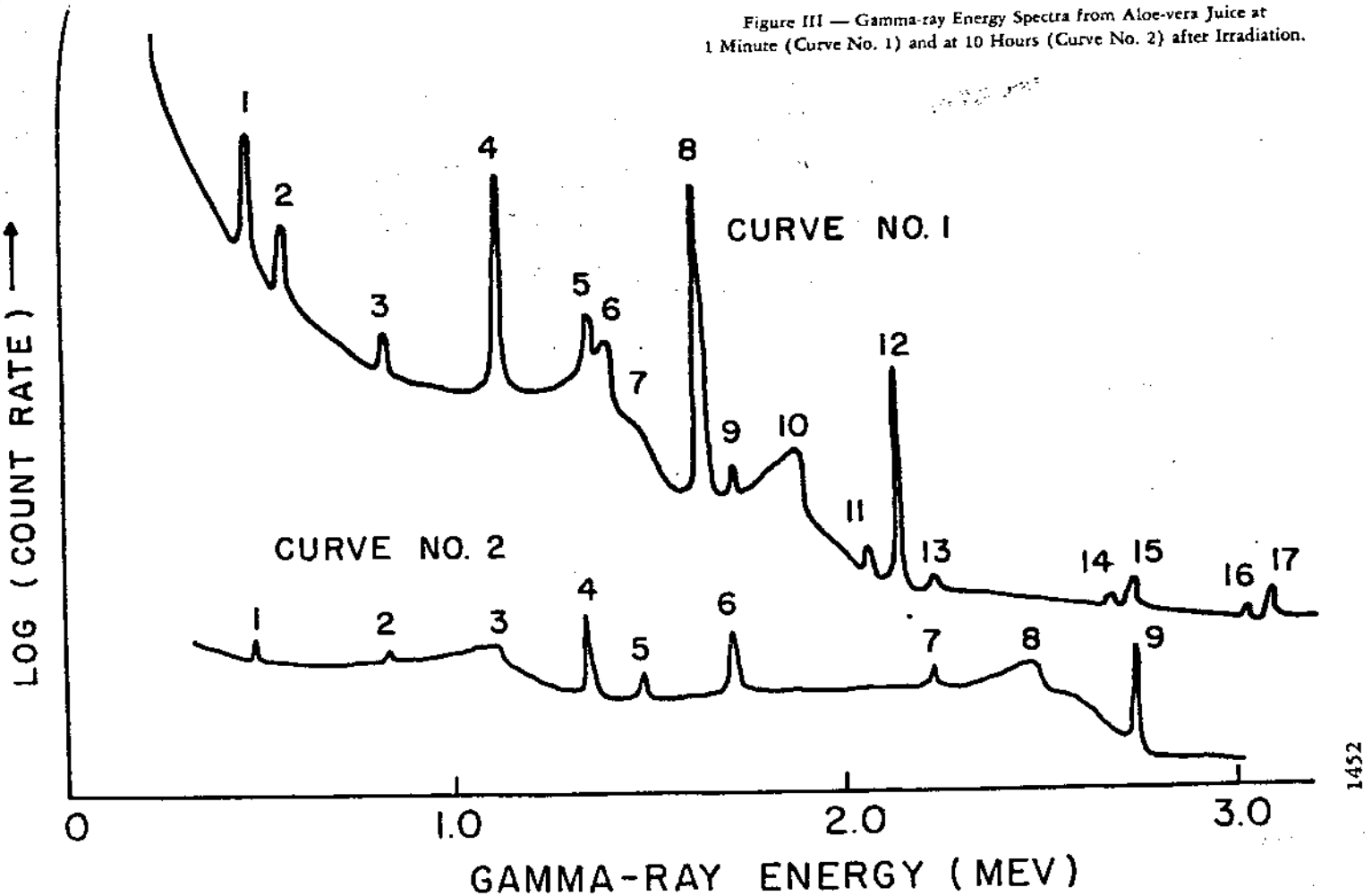
▨ Insensitive to thermal neutron activation, but sensitive to fast neutron activation analysis.

■ Not detectable by instrumental neutron activation analysis.

▩ Artificial or naturally radioactive element.

Figure 11. Sensitivity of Instrumental Neutron Activation Analysis Using a Lithium-drifted Germanium Detector System

Figure III — Gamma-ray Energy Spectra from Aloe-vera Juice at 1 Minute (Curve No. 1) and at 10 Hours (Curve No. 2) after Irradiation.



content appears lower than average. In the commercial juices, the actual concentrations would be $^1/_{200}$ of the above.

Based on our current knowledge of the disease-curing properties of inorganic ions, it is difficult to visualize any panacea-like qualities, which could logically be ascribable to the mineral constituents in *Aloe vera* juice.

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CHEMICAL EXAMINATION OF *LEUCAS CEPHALOTES*

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Sitosterol and its glucoside have been isolated from the air-dried shrub of Leucas cephalotes.

The plant, *Leucas cephalotes* (Labiatae) is reported to possess insecticidal activity and its flowers are efficacious [1]) against burns. The shrub is also said to check inflammations and cure jaundice and stomach troubles [2]). In view of its many-fold medicinal properties, systematic chemical examination of the whole plant was undertaken and the result of which is reported below: —

The whole plant (collected locally) was dried in shade (8 Kg.) and exhaustively percolated with alcohol (95 %) and the solvent distilled off. The residue thus obtained was successively extracted with hexane and ether (1 : 1). The hexane-ether extract yielded a middle layer, containing some shining needles which was separated and recrystallised from alcohol (0.228 mg.), m.p. 286°. It was insoluble in common organic solvents, responded to LB and Fehlings tests and was resistant to alkaline (20 %) hydrolysis indicating it to be a true O-glycoside. The glycoside on further purification from