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Final Report on the Safety Assessment of Aloe Andongensis Extract, Aloe Andongensis Leaf Juice, Aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Arborescens Leaf Protoplasts, Aloe Barbadensis Flower Extract, Aloe Barbadensis Leaf, Aloe Barbadensis Leaf Extract, Aloe Barbadensis Leaf Juice, Aloe Barbadensis Leaf Polysaccharides, Aloe Barbadensis Leaf Water, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, and Aloe Ferox Leaf Juice Extract[1]

Plant materials derived from the Aloe plant are used as cosmetic ingredients, including Aloe Andongensis Extract, Aloe Andongensis Leaf Juice, Aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Arborescens Leaf Protoplasts, Aloe Barbadensis Flower Extract, Aloe Barbadensis Leaf, Aloe Barbadensis Leaf Extract, Aloe Barbadensis Leaf Juice, Aloe Barbadensis Leaf Polysaccharides, Aloe Barbadensis Leaf Water, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, and Aloe Ferox Leaf Juice Extract. These ingredients function primarily as skin-conditioning agents and are included in cosmetics only at low concentrations. The Aloe leaf consists of the pericyclic cells, found just below the plant's skin, and the inner central area of the leaf, i.e., the gel, which is used for cosmetic products. The pericyclic cells produce a bitter, yellow latex containing a number of anthraquinones, phototoxic compounds that are also gastrointestinal irritants responsible for cathartic effects. The gel contains polysaccharides, which can be acetylated, partially acetylated, or not acetylated. An industry established limit for anthraquinones in aloe-derived material for nonmedicinal use is 50 ppm or lower. Aloe-derived ingredients are used in a wide variety of cosmetic product types at concentrations of raw material that are 0.1% or less, although can be as high as 20%. The concentration of Aloe in the raw material also may vary from 100% to a low of 0.0005%. Oral administration of various anthraquinone components results in a rise in their blood concentrations, wide systemic distribution, accumulation in the liver and kidneys, and excretion in urine and feces; polysaccharide components are distributed systemically and metabolized into smaller molecules. aloe-derived material has fungicidal, antimicrobial, and antiviral activities, and has been effective in wound healing and infection treatment in animals. Aloe barbadensis (also known as Aloe vera)–derived ingredients were not toxic in acute oral studies using mice and rats. In parenteral studies, the LD_{50} using mice was >200 mg/kg, rats was >50 mg/kg, and using dogs was >50 mg/kg. In intravenous studies the LD_{50} using mice was >80 mg/kg, rats was >15 mg/kg, and dogs was >10 mg/kg. The 14-day no observed effect level (NOEL) for the Aloe polysaccharide, acemannan, in the diet of Sprague-Dawley rats, was 50,000 ppm or 4.1 to 4.6 g/kg day^{-1}. In a 3-month study using mice, Aloe vera (extracted in ethanol) given orally in drinking water at 100 mg/kg produced reproductive toxicity, inflammation, and mortality above that seen in control animals. Aloe vera extracted in methanol and given to mice at 100 mg/kg in drinking water for 3 months caused significant sperm damage compared to controls. Aloe barbadensis extracted with water and given to pregnant Charles Foster albino rats on gestational days (GDs) 0 through 9 was an abortifacient and produced skeletal abnormalities. Both negative and positive results were found in bacterial and mammalian cell genotoxicity assays using Aloe barbadensis–derived material, Aloe Ferox–derived material, and various anthraquinones derived from Aloe. Aloin (an anthraquinone) did not produce tumors when included in the feed of mice for 20 weeks, nor did aloin increase the incidence of colorectal tumors induced with 1,2-dimethylhydrazine. Aloe-emodin (an anthraquinone) given to mice in which tumor cells had been injected inhibited growth of malignant tumors. Other animal data also suggest that components of Aloe inhibit tumor growth and improve survival. Various in vitro assays also demonstrated anticarcinogenic activity of aloe-emodin. Diarrhea was the only adverse effect of note with the use of Aloe-derived ingredients to treat asthma, ischemic heart disease, diabetes, ulcers, skin disease, and cancer. Case reports include acute eczema, contact urticaria, and dermatitis in individuals who applied Aloe-derived ingredients topically. The Cosmetic Ingredient Review Expert Panel concluded that anthraquinone levels in the several Aloe Barbadensis extracts are well understood and can conform to the industry-established level of 50 ppm. Although the phototoxicity anthraquinone components of Aloe plants have been demonstrated, several clinical studies of preparations derived from Aloe barbadensis plants demonstrated no phototoxicity, confirming that the concentrations of anthraquinones in such preparations are too low to induce phototoxicity. The characterization of aloe-derived ingredients from other species is not clear. In the absence of well-characterized derivatives, biological studies of these materials are considered necessary. The studies needed are 28-day

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dermal toxicity studies on Aloe Andongensis Extract, Aloe Andongensis Leaf Juice, Aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, and Aloe Ferox Leaf Juice (ingredients should be tested at current use concentrations). In Aloe-derived ingredients used in cosmetics, regardless of species, anthraquinone levels should not exceed 50 ppm. The Cosmetic Ingredient Review Expert Panel advised the industry that the total polychlorobiphenyl (PCB)/pesticide contamination of any plant-derived cosmetic ingredient should be limited to not more than 40 ppm, with not more than 10 ppm for any specific residue and that limits were appropriate for the following impurities: arsenic (3 mg/kg maximum), heavy metals (20 mg/kg maximum), and lead (5 mg/kg maximum).

INTRODUCTION

Aloe Andongensis Extract, Aloe Andongensis Leaf Juice, Aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Arborescens Leaf Protoplasts, Aloe Barbadensis Flower Extract, Aloe Barbadensis Leaf, Aloe Barbadensis Leaf Extract, Aloe Barbadensis Leaf Juice, Aloe Barbadensis Leaf Polysaccharides, Aloe Barbadensis Leaf Water, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, and Aloe Ferox Leaf Juice Extract are cosmetic ingredients derived from Aloe. This review presents information relevant to the safety of these Aloe-derived ingredients as considered by the Cosmetic Ingredient Review (CIR) Expert Panel.

The general Aloe CAS number is 8001-97-6. *Aloe barbadensis* extract has the CAS number 85507-69-3. As described in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), these ingredients function primarily as skin-conditioning agents or have no specific function listed. In most cases, the definitions of these ingredients are tautologies, e.g., Aloe Barbadensis Flower Extract is an extract of the flowers of *Aloe barbadensis*.

CHEMISTRY

Definition

Plant material derived from the aloe plant is characterized by its source (e.g., what part of the plant), the species of plant, the physical description of the material, and by the constituents found in the material.

The definitions of each ingredient, according to the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), are:

Aloe Andongensis Extract is the extract of the leaves of *Aloe' andongensis*.

Aloe Andongensis Leaf Juice is the liquid expressed from the leaves of *Aloe andongensis*.

Aloe Arborescens Leaf Extract is the extract of the leaves of *Aloe arborescens*.

Aloe Arborescens Leaf Juice is the juice expressed from the leaves of *Aloe arborescens*.

Aloe Arborescens Leaf Protoplasts are the protoplasts obtained form the leaves of *Aloe arborescens*.

Aloe Barbadensis Flower Extract is the extract of the flowers of *Aloe barbadensis*.

Aloe Barbadensis Leaf is a plant material derived from the leaves of *Aloe barbadensis*.

Aloe Barbadensis Leaf Extract is an extract of the leaves of *Aloe barbadensis*.

Aloe Barbadensis Leaf Juice is the juice expressed from the leaves of *Aloe barbadensis*.

Aloe Barbadensis Leaf Polysaccharides is the polysaccharide fraction isolated from the leaf of *Aloe barbadensis*.

Aloe Barbadensis Leaf Water is an aqueous solution of the odoriferous principles distilled from the leaves of *Aloe barbadensis*.

Aloe Ferox Leaf Extract is an extract of the leaves of *Aloe ferox*.

Aloe Ferox Leaf Juice is the juice expressed from the leaves of *Aloe ferox*.

Aloe Ferox Leaf Juice Extract is an extract of the juice of the leaf of *Aloe ferox*.

Table 1 gives a list of synonyms for these ingredients as given in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004). *Aloe vera* is a frequently used synonym for *Aloe barbadensis*.

Composition and Physical and Chemical Properties

Gjerstad (1969) described Aloe as the dried juice of the lower portion of the leaves of any three geographical varieties of the Aloe genus. The juice appears blackish-brown, opaque, and smooth.

Ghannam et al. (1986) described the solid residue of *Aloe barbadensis* (native to Mediterranean countries and the Saudi Arabian peninsula) obtained by evaporating the sap drained from the cut leaves. This drained latex solidifies and turns brown on exposure to air. It contains anthraquinone glycosides, barbaloin, and β-barbaloin, the hydrolysis of which yields aloe-emodin and *d*-arabinose.

Natow (1986) stated that Aloe is the name given to the genus that includes more than 300 plants, grown all over the world. This report considers ingredients from four species: *andongensis, arborescens, barbadensis*, and *ferox*.

Klein and Penneys (1988), Briggs (1995), and the M.D. Anderson Cancer Center (2003) described the leaf of Aloe plants as consisting of two main parts. One part, the pericyclic cells, is found just below the plant's skin. The pericyclic cells produce a bitter, yellow latex known as Aloe juice, or latex. When this juice dries it forms a dark brown solid material. The latex contains the anthraquinone, emodin, a gastrointestinal irritant responsible for cathartic effects. The second part, the inner central area of the leaf, contains the thin walled parenchymal cells that produce the clear slightly viscous (mucilaginous) fluid known as Aloe gel or inner gel. This gel contains the polysaccharides. There are generally more than one polysaccharide found in the

TABLE 1
List of synonyms (Gottschalck and McEwen 2004)

Ingredient	Synonyms
Aloe Andongensis Extract	Aloe Extract
	Extract of *Aloe andongensis*
Aloe Andongensis Leaf Juice	*Aloe andongensis*
Aloe Arborescens Leaf Extract	*Aloe arborescens*
	Kidachi Aloe
Aloe Arborescens Leaf Juice	*Aloe arborescens*
	Kidachi Aloe Kajyu
	Kidachi Aloe Youjyu
	Kidachi Aloe Youjyu Masto
Aloe Arborescens Leaf Protoplasts	*Aloe arborescens*
	Aloe arborescens CRS
Aloe Barbadensis Flower Extract	*Aloe barbadensis*
	Aloe barbadensis Extract
	Aloe Flower Extract
	Extract of *Aloe barbadensis* Flower
	Extract of Aloe Flowers
Aloe Barbadensis Leaf	Aloe
	Aloe barbadensis
	Aloe Leaf Powder
	Aloe vera
Aloe Barbadensis Leaf Extract	*Aloe barbadensis*
	Aloe Extract
	Barbados Aloe Extract
	Curacao Aloe Extract
	Extract of Aloe
	Extract of *Aloe barbadensis*
	Extract of Aloe leaves
	Aloe vera
Aloe Barbadensis Leaf Juice	*Aloe barbadensis*
	Aloe Gel
	Aloe barbadensis Gel
	Aloe Juice
	Aloe vera Gel
Aloe Barbadensis Leaf Polysaccharides	*Aloe barbadensis*
Aloe Barbadensis Leaf Water	*Aloe barbadensis*
Aloe Ferox Leaf Extract	*Aloe ferox*
	Extract of *Aloe ferox*
	Cape Aloe
Aloe Ferox Leaf Juice	*Aloe ferox*
	Cape Aloe
Aloe Ferox Leaf Juice Extract	*Aloe ferox*
	Cape Aloe Ekisu

gel; they can be acetylated, partially acetylated, or not acetylated. The gel portion (Aloe leaf juice) is the part of the plant generally used for emollient and moisturizing effects desired in cosmetics, and the healing action desired for certain medicinal products.

The International Aloe Science Council (IASC) distinguished between the anthraquinone found in the outer cell layer of the aloe leaf and the rest of the plant (IASC 2003). According to this group, the maximum allowable aloin content in aloe-derived material for nonmedicinal use is 50 ppm or lower.

TABLE 2
Description of Aloe products (Carrington Labs 2001)

Product name	Appearance	Odor	Taste	pH
Aloe vera Whole Leaf 1:1	Light amber liquid	Light vegetable	Slight bitter vegetable	3.0–4.0
Aloe vera gel 1:1 decolorized	Clear, slightly translucent	Light vegetable	Slight characteristic vegetable	3.5–5.0
Aloe vera gel 10:1 decolorized	Semiviscous, light to medium amber	Light vegetable	Slight vegetable	3.5–5.0
Aloe vera gel 40:1 decolorized	Semiviscous, light to medium amber	Light vegetable	Slight vegetable	3.5–5.0
Aloe vera Gel Freeze Dried Powder 200:1	White to light tan powder	Not given	Not given	Not given

Danhof (1998) stated that aloe polysaccharides consist of linear chains of β-1-4-linked glucose and mannose molecules, in which mannose predominates. Chain lengths may be as small as from a few molecules up to several thousand, but by convention the lower end of the molecular weight is usually considered to be 1000 daltons, consistent with being a polysaccharide. Depending on the particular chain length, this material will have different physical properties.

The IASC (1998) maintains a certification program in which "whole leaf aloe vera gel" is expected to adhere to the following specifications: solids between 0.46 to 1.31%; pH between 3.5 and 4.7; calcium between 98.2 and 448 mg/L; magnesium between 23.4 and 118 mg/L; and malic acid between 817.8 and 3427.8 mg/L. Consistent with these specifications, Rowe and Parks (1941) determined the pH of fresh *Aloe vera* leaves to be 4.7.

Table 2 presents descriptions and chemical properties of commercial Aloe products from one company analyzed by Carrington Labs (2001). All products listed in the table are considered acceptable for use in cosmetics, and are derived from *Aloe barbadensis*.

Active Organics (2002) described a trade name product, Actiphyte, of Aloe Vera 10 Fold, as consisting of Propylene Glycol and Aloe Barbadensis Leaf Extract. The product was a clear to pale yellow liquid, with a characteristic odor, and a flash point of 228°F, boiling point of 225°F, specific gravity of 1.02 to 1.05 (at 25°C), complete solubility in water, refractive index of 1.3620 to 1.3700 (at 25°C), and a pH of 4.0 to 6.5 (at 25°C).

As noted earlier, aloe-derived ingredients also are characterized by their specific constituents. The constituents reported by Shelton (1991) and Vogler and Ernst (1999) found in *Aloe vera* are listed in Table 3.

TABLE 3
Constituents of Aloe Vera (Shelton 1991; Vogler and Ernst 1999)

Anthraquinones	Saccharides	Vitamins	Nonessential amino acids	Inorganic compounds	Enzymes	Essential amino acids	Miscellaneous
Aloin	Cellulose	B_1	Histidine	Calcium	Cyclooxygenase	Lysine	Cholesterol
Barbaloin	Glucose	B_2	Arginine	Sodium	Oxidase	Threonine	Triglycerides
Isobarbaloin	Mannose	B_6	Hydroxyproline	Chlorine	Amylase	Valine	Steroids
Anthranol	L-Rhamnose	Choline	Aspartic Acid	Manganese	Catalase	Leucine	β-Sitosterol
Aloetic Acid	Aldopentose	Folic Acid	Glutamic Acid	Zinc	Lipase	Isoleucine	Lignins
Cinnamic Acid Ester		C	Proline	Chromium	Alkaline phosphatase	Phenylalanine	Uric Acid
Aloe-emodin		α-Tocopherol	Glycine	Copper	Carboxypeptidase	Methionine	Gibberellin
Emodin		β-Carotene	Alanine	Magnesium			Lectin-like substance
Chrysophanic Acid			Tyrosine	Iron			Salicylic Acid
Resistannol							Arachidonic Acid
Anthracene							Potassium Sorbate
Ethereal Oil							

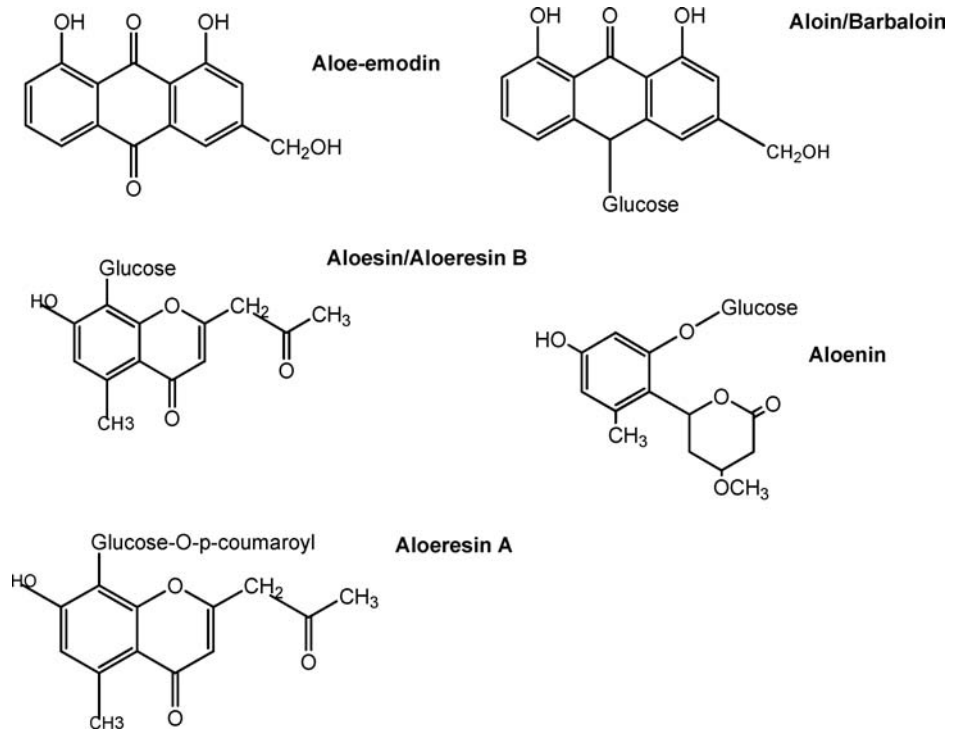

FIGURE 1
Structures of the main anthraquinone compounds of Aloe species (Suga and Hirata 1983; van Wyk et al. 1995).

Mabusela et al. (1990) stated that the glucomannans of *Aloe ferox* are mainly arabinogalactan and rhamnogalactan. According to van Wyk et al. (1995), aloesin, aloeresin a, and Aloin make up 70% to 98% of the total dry weight of *Aloe ferox* leaf exudate. A description and some physical and chemical properties of these and other compounds present in the various Aloe species are found in Table 4.

Figure 1 shows the chemical structures of anthraquinones and anthraquinone derivatives of *Aloe barbadensis, Aloe arborescens,* and *Aloe ferox* (Suga and Hirata 1983; van Wyk et al. 1995). These compounds are generally found in the pericyclic cells but can be found within the gel as well (see Methods of Manufacture and Impurities).

Ultraviolet Absorption

Proserpi (1976) examined the ultraviolet (UV) absorption of various Aloe species and aloin, a component of Aloe species. Both Aloe extracts and aloin have spectrophotometric peaks at about 297 nm. The aloin spectrogram has a second peak at 360 nm. The author concluded that cosmetics containing 1% to 2% of aloe extract should give effective sunburn protection due to the substances contained within Aloe performing selective screening of UV radiation, by absorbing mainly in the erythemogenic rays.

Bader et al. (1981) examined dry extracts of Aloe (containing anthraquinones) for their ability to absorb light in the UVB range. Extraction was done with a 50% water–ethyl alcohol solution. The aloe extracts had maximum absorption around 294 nm. The coefficient of extinction for a glycolic extract of *Aloe vera* was 73.8 at 308 nm and 85.0 at 295 nm (Bobin et al. 1994). Absorption and fluorescence emission spectra of aloe emodin in ethanol yielded a maximum absorption wavelength of 451 nm and a maximum fluorescence emission peak at 537 nm (Vargas et al. 2002).

Absorption and fluorescence emission spectra of aloe emodin in acetonitrile yielded an absorption spectrum with a maximum at 430 nm. The fluorescence emission spectrum for aloe emodin at 430 nm exhibits with emissions peaks at 565, 530, and 495 nm. Increasing the fraction of water increased the intensity of the 565 and 530 nm fluorescence peaks relative to the 495 nm peak. Aloe emodin was photostable at UVA radiation irradiances of 2.9×10^{-3} W/cm^2 (Vath et al. 2002).

Methods of Manufacture

According to Grindlay and Reynolds (1986), commercial cultivation of *Aloe vera* for its gel began in the 1920s in Florida. Current cultivation in the United States primarily takes place in Florida, Texas, and Arizona. *Aloe vera* can be harvested by hand, with the leaves cut off at the base of the plant. Individual leaves are wrapped, crated, and transported to processing plants where they are cleaned. Next the outer layers are removed by filleting; this allows the removal of the central filet of gel. Once the gel is

TABLE 4
Physical and chemical properties of aloe constituents

Component	Chemical ID	MW	UV absorption peaks	Melting point	Description	Reference
Acemannan	β-(1,4) acetylated polymannose with interspersed *O*-acetyl groups aka Carn 750, Carn 1000, Polymannoacetate, Aliminase, Alovex, Carrisyn	80,000	—	—	Long chain, polydispersed	McAnalley 1990
Acetylated Mannan	—	40,000	—	—	—	Yagi et al. 1977
Aloctin A	Lectins	18,000	—	—	—	Suzuki et al. 1979
Aloctin B		24,000	—	—	—	
Aloe-emodin	1,8-Dihydroxy-3-hydroxymethyl-9,10-anthracenedione	270.23	221, 253, 266, 289, 483 nm in ethanol	220–221	Water soluble; depending on the concentration color can be red through brown to black	Hirata and Suga 1977; Budavari 1989; Strickland et al. 2000
	1,8-Dihydroxy-3-(hydroxymethyl)-9,10-anthracenedione					
	1,8-Dihydroxy-3-hydroxymethylanthraquinone					
	3-Hydroxymethylchrysazin, 1,8-dihydroxy-3-(hydroxymethyl)anthraquinone					
	1,8-Dihydroxy-3-hydroxymethyl-anthraquinone					
	$C_{15}H_{10}O_5$					
Aloeferon	—	70,000	—	—	—	Madis et al. 1989
Aloemannan	Glucomannan	374,000	—	—	—	Gowda et al. 1979
Aloe Mannan	Partially acetylated β-D-mannan	15,000	—	—	Water soluble	Yagi et al. 1977
Aloenin (aloearbonaside)	4-Methoxy-6[10-hydroxy-12-methyl-8-*O*-D-glucopyranosyl]phenyl-pyrone-2	—	232, 247, 307 in ethanol; 302 in methanol	145–147	Bitter glucoside	Suga and Hirata 1972; Makino et al. 1973; Hirata and Suga 1977; Hirata et al. 1981; Gutterman 2000
	O-glucoside					
	$C_{19}H_{22}O_{10}\cdot H_2O$					
Aloeresin B	Synonym for Aloesin	—	—	—	—	Makino et al. 1973
Aloeresin A	5-Methylchromone C-glycoside	—	—	148–150	*O*-*p*-coumaroyl derivative of Aloesin	Gramatica et al. 1982
Aloeride	Polysaccharide	4–7 $\times$ 10^6	—	—	Soluble white powder	Madis et al. 1989

Aloesin	2-Acetylonyl-8-β-D-glucopranyosyl-7-hydroxy-5-methylchromone C-glucosyl chromone $C_{17}H_{18}O_9$	394	248, 254, 297 in ethanol; 216, 340 in methanol	142–144	Water soluble	McCarthy and Haynes 1967; Holdsworth 1971; Hirata and Suga 1977; van Wyk et al. 1995; Gutterman 2000
Aloin	10β-D-glucopryanosyl-1,8-dihydroxy-3-hydroxymethyl-9(10H) anthracenone $C_{21}H_{22}O_9$	418.36	250–290 in methanol, peak at 260	148–149	Bitter juice that dries to a yellow powder; can be hydrolyzed to aloe-emodin; includes Aloin A and Aloin B diastereomers; slightly soluble in water and organic solvents	Hay and Haynes 1956; Joint Committee 1967; McCarthy 1969; Hirata and Suga 1977; Budavari 1989; Shelton 1991; van Wyk et al. 1995; Koch, 1996; Viljoen et al. 2001
Aloe species extract; aka Aloe species resin	Dried Aloe barbadensis Miller or Aloe ferox Miller	—	—	—	Water extracted; brown fine powder; tapped density 0.5g/ml; particle size not less than 90% <300 μm	Hammer Pharma 2002
Arborans A	Glycans with a small amount of *O*-acetyl groups	1.2×10^4	—	—	—	Hikino et al. 1986
Arborans B		5.7×10^4				
Verectin	Glycoprotein	29,000	—	—	—	Yagi et al. 1997, 2000

removed, the cell walls, lignified fibers, and other contaminants are removed by either squeezing and filtering or by a decantation process. When removing the gel, care needs to be taken to not contaminate the gel with the green rind. Because the gel's activity becomes unstable after removal from the leaves, a number of processes have been developed to overcome this instability. One method used to stabilize the gel is to expose the gel to high temperatures for a short time (3 min). Ultraviolet stabilization, chemical oxidation with hydrogen peroxide, and preservatives and additives are other methods of retaining the gel's activity.

A method of manufacture patented by McAnalley (1990) isolates the gel filet of *Aloe vera* and can further isolate the active ingredient carrisyn (also known as acemannan) (see Table 3). According to this method, the leaf, cut from the base of the Aloe plant, is washed in a bactericidal solution and the end portion of the leaf is removed to allow the anthraquinone-rich sap to drain out, additionally the rind is removed. McAnalley's method also allows for the leaf of the plant to be crushed and then dialyzed to remove the anthraquinones. The gel filet that is left behind is ground and homogenized to produce aloe juice. The juice is then filtered to remove fibrous material. At this point the juice can have preservatives, flavors, excipient carriers, and/or colorant added to it. To further isolate the active ingredient, an aliphatic polar solvent is added to precipitate out carrisyn. Carrisyn is then sterilized and eventually dried by lyophilization.

The process used by Aloecorp involves soaking the just harvested leaves in a food-grade sanitizer to reduce microbial counts, the gel is then extracted, flash cooled to 5°C, and then concentrated while under vacuum. The concentrated gel is then freeze dried (Aloecorp 2001).

Agarwala (1997) stated that the primary difference between whole leaf processing versus inner gel processing from the whole leaf is that for the inner gel, leaves are manually or machine filleted before further processing. In both processes, the whole leaf is cut and quickly transported to the processing center. After washing, the whole leaves are either ground through a hammer mill and directed into a heating vat, or filleted to remove the inner gel. Heating is done to reduce the amount of slime. Heating can take place one of two ways: at low temperature for a long time, or at high temperature for a short time. After heating, the slurry is filtered through a screw press or cloth press. After partial cooling takes place, activated charcoal is added (decolorizes and adsorbs the bitter components). For complete clarity, the gel is filtered through diatomaceous earth.

For the inner gel, this process results in 0.5% solids, of which 0.1% are large molecules such as polysaccharides, glycoproteins, and proteins, and 0.4% of which are small molecules such as simple sugars, organic and inorganic salts, and nitrogenous compounds. For the whole leaf, the process results in 1.5% solids, of which 0.05% are large molecules and 1.45% are small molecules. The practical ramification of these two techniques is that for each ton of whole leaves, processing the whole leaf could produce around 25 to 30 pounds of total soluble solids from the gel component, while filleting the leaf before processing could produce around 5 pounds. The author states that some manufacturers do process the whole leaf, taking care in the process to remove bitter anthraquinones from the final product.

Table 5 shows the different properties of material derived from the inner gel versus the whole leaf (Agarwala 1997).

Meadows (1980) used 2% sodium benzoate and 0.15% ascorbic acid to preserve aloe gel. Agarwala (1997) stated that the most commonly used preservatives are sodium benzoate and potassium sorbate with pH adjusted to <4.6 with citric acid.

TABLE 5
Comparison of inner gel and whole leaf material (Agarwala 1997)

Property	Inner gel	Whole leaf
Soluble solids	0.62%	1.3–3.5%
pH	4.5	4.2
Nitrogen (as ammonia)	12	6
Ca^{2+} (mg/L)	340	600
Mg^{2+} (mg/L)	60	100
K^{+} (mg/L)	390	750
Conductivity	1200–2300 μ	1900–2500 μ
Taste	Bland	Salty
Methanol precipitable solids (MPS)	0.12–0.16%	0.45–1.3%
Ratio of MPS to total solids	25–35	30–40
Ratio of ethanol precipitable solids to total solids	6–10	1–10
HPLC peaks	E-peak	E-peak; unidentified peak at 21–31 min
UV absorption peak	224 nm	254 nm
Monosaccharide	Galactan and glucomannan	Glucomannan

Aloe extracts and aloe gel extracts can be freed of anthraquinones by means of activated charcoal and filleting processes (UNITIS 2003).

Aloe ferox suitable for cosmetic purposes can be manufactured by two methods: (1) percolation with hot water at 60°C and concentrated under vacuum to dryness, or (2) percolation with propylene glycol and concentrated under vacuum (Patri and Silano 2002).

Analytical Methods

Nakamura and Okuyama (1990) detected aloenin, a component of *Aloe arborescens,* in cosmetics (lotions and creams) by gas chromatography with mass fragmentography. Kuzuya et al. (2001) determined the presence of aloenin, barbaloin, and isobarbaloin (all components of *Aloe arborescens*) in commercial products using micellar electrokinetic chromatography. Reverse-phase high-performance liquid chromatography (HPLC) and paper or thin-layer chromatography (TLC) have been used to detect barbaloin content within Aloe plants (Ishii et al. 1984).

Reverse-phase HPLC and paper or thin-layer chromatography have been used to detect the various components of *Aloe barbadensis* (Hutter et al. 1996; Holdsworth 1971). Ross et al. (1997) used size exclusion chromatography to examine various products (cosmetic and non-cosmetic) for the levels of *Aloe vera* mucilaginous polysaccharide.

According to the IASC (2001a), polymerase chain reaction for the detection of Aloe DNA can allow for identification of plant species.

Leaf exudate samples from *Aloe ferox* were collected by applying slips of filter paper to a cut leaf and were analyzed by HPLC. The major leaf exudate compounds detected were aloesin, aloeresin, aloin B, aloin A, aloinoside B, and aloinoside A (Viljoen et al. 2001). Van Wyk et al. (1994) analyzed commercial samples (type not described) by HPLC and found the concentration of *Aloe ferox* constituents in the products were not consistent.

Waller et al. (1978) showed that *Aloe barbadensis* leaves contain various amino acids, with highest concentration of arginine (449 μmol/100 g), followed by asparagine (344 μmol/100 g), glutamate (294 μmol/100 g), aspartate (237 μmol/100 g), and serine (224 μmol/100 g). The authors also found that mannose and glucose were in a molar ratio of 5:4 and trace amounts of xylose, rhamnose, galactose, and either arabinose or fructose were also present.

Robson et al. (1982) performed a detailed chemical analysis of 99.5% pure *Aloe vera* extract and found organic compounds such as glucose (13 mg/dl), uric acid (0.5 mg/dl), salicylic acid (3.6 mg/dl), creatinine (1.9 mg/dl), alkaline phosphatase (1 IU/L), creatinine phosphokinase (10 IU/L), cholesterol (11 mg/dl), triglycerides (374 mg/dl), lactate (14.8 mg/dl), and protein (0.2 mg/dl). Inorganic constituents found were sodium (19 mEq/L), potassium (21.5 mEq/L), inorganic phosphorus (14 mg/dl), and chloride (1 mEq/L). Trace metals present in the extract were calcium (23.5 mEq/L), magnesium (4.6 mg/dl), copper (0.2 mg/dl), and zinc (0.02 mg/dl). Aloe contains a number of anthraquinone glycosides, the principal one of which is barbaloin (aloe-emodin anthrone C-10 glucoside) (Tyler et al. 1988). O-Glycosides of barbaloin with an additional sugar also have been isolated from certain samples of Cape aloe. These compounds have been designated aloinosides. Free (nonglycosidal) aloe-emodin and a free and combined anthranol are also present. Chrysophanic acid has been detected in certain types of aloe. The active constituents of aloe vary qualitatively and quantitatively according to the species from which the material is obtained. In addition to the physiologically active compounds (10% to 30%), aloe contains inactive ingredients including large amounts (16% to 63%) of a resinous material plus a volatile oil.

Aloe-emodin can be determined using HPLC and ranges between 2.9 and 3.7 ppm in *Aloe arborescens* (UNITIS 2003).

Impurities

Danof and McAnalley (1983) were able to show that the yellow sap (containing the anthraquinones) was lethal in human fibroblast assays; care should be taken to keep it out of commercial products. Examples of anthraquinones and anthraquinone derivatives include aloin, aloe-emodin, aloesin/aloeresin B, aloeresin A, aloin/barbaloin, and aloenin.

CTFA (2002) reported a study where the amount of aloe-emodin present in Aloe Arborescens CRS was determined. Aloe Arborescens CRS was filtered to remove insoluble components and the liquid component was analyzed using high pressure liquid chromatography and compared to a standard aloe-emodin solution. It was found that samples 1 and 2 from the filtered Aloe Arborescens CRS contained an average (of two runs) of 2.9 and 3.7 ppm aloe-emodin, respectively.

The International Aloe Science Council (2001b) considers the presence of maltodextrin, lactobacillus, lactic acid, or acetic acid to be a sign of bacterial contamination of commercial products.

Nakano et al. (1985) detected various levels of aloenin in 15 commercial cosmetic lotions by HPLC analysis. Table 6 lists the results of the HPLC analysis on 5 μl of the commercial lotions. Nakamura and Okuyama (1990) detected the anthraquinone aloenin in several commercial skin care lotions, creams, etc. (0.5-g samples). The average recoveries were 94% with a relative standard deviation of 4% to 7% and a detection limit of 0.02 μg/g. The aloenin content of the cosmetic products are shown in Table 7.

The values in Tables 6 and 7 may be compared with the IASC (1998) stated limit of 50 ppm (equivalent to μg/ml and μg/g in the tables).

Kim et al. (1998) developed simple and accurate methods to detect adulteration of commercial Aloe gel products by other plant polysaccharides. Isolation of crude polysaccharides from

TABLE 6
Aloenin levels in commercial lotions (Nakano et al. 1985)

Product	Aloenin (μg/ml)
A	32.4
B	17.7
C	4.8
D	1.2
E	13.0
F	0.8
G	0.8
H	0.7
I	0.6
J	0.5
K	0.4
L	0.3
M	<0.2
N	<0.2
O	<0.2

commercial aloe gel powders was performed by precipitation with ethyl alcohol. Analyses of free sugars was by gas chromatography and quantification of total hexose was by the DuBois assay. Maltodextrin was detected by two methods, TLC and HPLC (maltodextrin in Aloe products is considered to be a contaminant).

^{1}H nuclear magnetic resonance (NMR) can be used to detect the presence of lactic acid, acetic acid, and formic acid in *Aloe vera* products. All three acids are undesirable ingredients in *Aloe vera* products (Diehl and Teichmuller 1998).

TABLE 7
Aloenin levels in commercial products (Nakamura and Okuyama 1990)

Sample	Aloenin content (μg/g)
Lotion 1	42
Lotion 2	1.2
Lotion 3	0.80
Milky Lotion 1	1.7
Milky Lotion 2	0.96
Cream 1	2.3
Cream 2	3.5
Cream 3	4.0
Cream 4	0.25
Cream 5	1.8
Face Pack 1	2.3
Face Pack 2	4.8
Hair Rinse	1.6
Hair Tonic	6.2

USE

Cosmetic

As given in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004), Aloe Andongensis Extract and Aloe Andongensis Leaf Juice function as a skin-conditioning agent—humectant; Aloe Arborescens Leaf Juice, Aloe Barbadensis Flower Extract, Aloe Barbadensis Leaf Juice, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, Aloe Ferox Leaf Juice Extract and Aloesin all function as skin-conditioning agents—miscellaneous in cosmetic formulations. Aloe Barbadensis Leaf Extract functions as an external analgesic, humectant, oral care agent, skin-conditioning agent—miscellaneous, and skin-conditioning agent—emollient in cosmetic products. Aloe Barbadensis Leaf Polysaccharides functions as film former, humectant, skin-conditioning agent—emollient, and skin conditioning agent—humectant. Aloe Barbadensis Leaf Water functions as a fragrance ingredient. The function of Aloe Arborescens Leaf Extract and Aloe Barbadensis Leaf in cosmetic formulations was not reported.

The frequency of Aloe use reported to the Food and Drug Administration (FDA) in 2001 is shown in Table 8 (FDA 2001). Unfortunately, the terminology of aloe-derived ingredients in the categories in the FDA database do not correspond to those used by industry (CTFA 2002). To merge the two pieces of information, the following assumptions were made: (1) Aloe Extract reported to FDA meant Aloe Barbadensis Leaf Extract; (2) Aloe reported to FDA meant Aloe Barbadensis Leaf; (3) Aloe Arborescens reported to FDA meant Aloe Arborescens Leaf Extract; (4) Aloe Flower Extract reported to FDA meant Aloe Barbadensis Flower Extract; (5) Aloe Gel reported to FDA meant Aloe Barbadensis Leaf Juice; Aloe, Powdered reported to FDA meant all ingredients referred to as powders in the CTFA (2002) submission; and (6) that there was nothing reported to FDA comparable to the current industry data on Aloe Ferox Leaf Extract.

According to the Japan Ministry of Health, Labor and Welfare (MHWL), the Aloe ingredients reviewed in this report are not included on the list of ingredients that must not be combined in cosmetic products that are marketed in Japan (MHLW 2001a) or on the restricted ingredient list for cosmetic products that are marketed in Japan (MHLW 2001b). In addition, none of the Aloe ingredients found in this report are restricted from use in any way under the rules governing cosmetic products in the European Union (European Commission 2002).

Noncosmetic

The FDA has determined that *Aloe perryi, Aloe barbadensis, Aloe ferox*, and hybrids of this species with *Aloe africana* and *Aloe spicata* are food additives permitted for direct addition to food for human consumption as natural flavoring substances (21 CFR 172.510).

According to Duke and Beckstrom-Sternberg (1994) the Flavor and Extract Manufacturers' Association acceptable level for *Aloe vera* was 5 to 2000 ppm.

TABLE 8
Product formulation data for aloe-derived ingredients

Product category (number of formulations in each category) (FDA 2002)	Formulations containing ingredient (FDA 2002)	Concentration of raw material in product (%)[a] (CTFA 2002)	Concentration of Aloe in raw material (% solids) (CTFA 2002)
	Aloe[b]		
Baby lotions, oils, powders, and creams (60)	2	—	—
Other Baby Products (34)	1	—	—
Bath Oils, Tablets, and Salts (143)	3	—	—
Bubble Baths (215)	2	—	—
Other Bath Preparations (196)	7	—	—
Eyeliner (548)	3	—	—
Eye Lotion (25)	1	—	—
Eye Makeup Remover (100)	1	—	—
Other Eye Makeup Preparations (152)	3	—	—
Other Fragrance Preparations (173)	1	—	—
Hair Conditioners (651)	7	—	—
Shampoos (Non-Coloring) (884)	7	—	—
Hair Tonics, Dressings, etc. (598)	1	—	—
Other Hair Preparations (277)	1	—	—
Blushers (All Types) (245)	6	—	—
Face Powders (305)	3	—	—
Lipstick (962)	1	—	—
Makeup Bases (141)	8	—	—
Bath Soaps and Detergents (421)	7	0.0005	100
Deodorants (Underarm) (247)	3	—	—
Feminine Deodorants (4)	2	—	—
Other Personal Cleanliness Products (308)	2	0.05	200 × (45% solids)[c]
Aftershave Lotion (231)	7	—	—
Shaving Cream (134)	6	—	—
Other Shaving Preparation Products (63)	7	—	—
Skin Cleansing Creams, Lotions, etc. (775)	10	0.01	100
Depilatories (34)	2	—	—
Face and Neck Skin Care Preparations (310)	5	0.05	100
Body and Hand Skin Care Preparations (840)	11	0.05	100
Body and Hand Sprays (35)	—	0.01	0.25
Moisturizers (905)	24	—	—
Night Creams, Lotions and Powders (200)	2	—	—
Paste Masks (Mud Packs) (271)	3	—	—
Skin Fresheners (184)	4	—	—
Other Skin Care Preparations (725)	15	0.04	100
Suntan Gels, Creams, and Liquids (131)	9	—	—
Indoor Tanning Preparations (71)	4	—	—
Total	181	0.005–0.05	
	Aloe Extract[b]		
Baby Lotions, Oils, Powders, and Creams (60)	2	0.009–0.1	Not available
		0.1	5
		1	1×[c]
		0.1	40×[c]

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TABLE 8
Product formulation data for aloe-derived ingredients *(Continued)*

Product category (number of formulations in each category) (FDA 2002)	Formulations containing ingredient (FDA 2002)	Concentration of raw material in product (%)[a] (CTFA 2002)	Concentration of Aloe in raw material (% solids) (CTFA 2002)
Other Baby Products (34)	1	0.01	40×[c]
Bath Oils, Tablets, and Salts (143)	3	0.005	Not available
Bubble Baths (215)	2	0.2	4
Other Bath Preparations (196)	7	0.1–0.5	0.6–4
Eyeliner (548)	3	0.1	5
Eye Shadow (576)	6	0.05–0.1	Not available
		0.05–2	4–5
		0.2–1	1 × (0.5 – 2% solids)[c]
Eye Lotion (25)	1	0.1	5
Eye Makeup Remover (100)	1	—	—
Mascara (195)	—	0.1	5
Other Eye Makeup Preparations (152)	3	0.1–1	Not available
		0.001–0.2	4–5
Colognes and Toilet Waters (684)	—	0.3	Not available
		3	0.007
		0.3	4
Powders (273)	—	0.009–0.1	Not available
Other Fragrance Preparations (173)	1	0.5	4
Hair Conditioners (651)	7	6	0.007
		0.2–0.5	0.3–4
		0.05	50 (0.03% solids)[c]
		0.01	1 × (0.05–1% solids)[c]
Hair Sprays (Aerosol Fixatives) (275)	—	6	0.007
		0.2	4
		0.05	50 (0.03% solids)[c]
		0.0005	1 ×[c]
Hair Straighteners (63)	—	2	0.5
		0.05	50 (0.03% solids)[c]
Rinses (Non-Coloring) (42)		0.1	0.007
Shampoos (Non-Coloring) (884)	7	0.1	0.6
		0.01–0.5	3–4
		0.05	50 (0.03% solids)[c]
		0.0001–0.01	1 × (0.05–1% solids)[c]
Hair Tonics, Dressings, etc. (598)	1	0.001–0.2	0.6–5
Other Hair Preparations (277)	1	0.003	0.5
		0.01	50 (0.005% solids)[c]
Blushers (All Types) (245)	6	0.04–0.05	Not available
		0.1	5
Face Powders (305)	3	0.01	Not available
		0.1	5
		0.1–0.2	1 × (0.5–2% solids)[c]
Foundations (324)	—	0.07–0.3	Not available
		0.01–0.5	0.2–0.6
		0.02–0.2	4–5
		0.2	1 × (2% solids)[c]

TABLE 8
Product formulation data for aloe-derived ingredients *(Continued)*

Product category (number of formulations in each category) (FDA 2002)	Formulations containing ingredient (FDA 2002)	Concentration of raw material in product (%)[a] (CTFA 2002)	Concentration of Aloe in raw material (% solids) (CTFA 2002)
Lipstick (962)	1	0.4–6	Not available
		0.1–5	0.6–5
		1–6	25
		0.3	1 × (2% solids)[c]
Makeup Bases (141)	8	0.2	Not available
		0.5	4
		0.2	1 × (2% solids)[c]
Rouges (28)	—	1	Not available
Other Makeup Preparations (201)	—	2	Not available
		0.001	4
Cuticle Softeners (19)	—	0.01	4
Nail Polish and Enamel (123)	—	0.0005	Not available
Other Manicuring Preparations (55)	—	0.03	Not available
		5	97
Bath Soaps and Detergents (421)	7	0.1	0.007
		0.05	1 (0.0005% solids)[c]
		0.01–0.5	4–5
		0.1	1 × (0.05–1% solids)[c]
Deodorants (Underarm) (247)	3	<0.01–0.5	2–4
		0.1	40 × (16% solids)[c]
Feminine Deodorants (4)	2	—	—
Other Personal Cleanliness Products (308)	2	0.1	0.007
Aftershave Lotion (231)	7	0.5	0.6
		0.3	4
Preshave Lotion (all types) (14)	—	0.1	0.6
Shaving Cream (134)	6	0.0003	Not available
		0.003–1	0.6–4
Other Shaving Preparation Products (63)	7	0.05	Not available
		0.2	0.6
Skin Cleansing Creams, Lotions, etc. (775)	10	0.002–0.5	0.6–4
		0.05	1 (0.0005% solids)[c]
		0.0005	1×[c]
		0.01	40×[c]
Depilatories (34)	2	0.1	0.6
Face and Neck Skin Care Preparations (310)	5	0.00009–1	0.6–4
Body and Hand Skin Care Preparations (840)	11	0.03	Not available
		0.1–3	0.1–4
		0.1	1 (0.001% solids)[c]
Body and Hand Sprays (35)	—	0.06	0.6
Moisturizers (905)	24	0.1–0.5	0.6–4
		0.4	40
Night Creams, Lotions, and Powders (200)	2	0.1–0.5	0.6–4
Paste Masks (Mud Packs) (271)	3	0.5	4
		1	50 (0.5% solids)[c]
Skin Fresheners (184)	4	0.0006–0.5	0.3–4
		0.05	97

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TABLE 8
Product formulation data for aloe-derived ingredients *(Continued)*

Product category (number of formulations in each category) (FDA 2002)	Formulations containing ingredient (FDA 2002)	Concentration of raw material in product (%)[a] (CTFA 2002)	Concentration of Aloe in raw material (% solids) (CTFA 2002)
Other Skin Care Preparations (725)	15	0.05	Not available
		0.1	0.6
		0.007	7
		0.05	10×[d]
Suntan Gels, Creams, and Liquids (131)	9	0.1	1 (0.7–1.2% solids)[c]
		0.05	25
Indoor Tanning Preparations (71)	4	—	—
Other Suntan Preparations (38)	3	2	5
		0.3	25
Total	190	0.00009–6	
	Aloe Arborescens[b]		
Moisturizers (905)	1	—	—
Paste Masks (Mud Packs) (271)	—	0.3	5
Total	1	0.3	
	Aloe Flower Extract[b]		
Hair Conditioners (651)	—	0.05	10
Shampoos (Noncoloring) (884)	—	0.05	10
Hair Dyes and Colors (1690)	—	0.05	5
Bath Soaps and Detergents (421)	—	0.05	0.5
Skin Cleansing Creams, Lotions, etc. (775)	1	0.01	0.2–0.6
Total	1	0.01 – 0.05	
	Aloe Gel[b]		
Baby Shampoos (29)	—	1	1 × (0.5% solids)[c]
Baby Lotions, Oils, Powders, and Creams (60)	4	1–2	1 × (0.5–2% solids)[c]
Other Baby Products (34)	4	—	—
Bath Oils, Tablets, and Salts (143)	2	0.1	1 × (0.5% solids)[c]
Bubble Baths (215)	18	0.1	1 × (0.5% solids)[c]
Other Bath Preparations (196)	4	1	100 (0.5% solids)[c]
Eyebrow Pencil (102)	—	0.1	100 (0.5% solids)[c]
Eyeliner (548)	—	0.1–1	
Eye Shadow (576)	3	—	—
Eye Lotion (25)	—	2	1 × (0.5% solids)[c]
Eye Makeup Remover (100)	4	1–2	1 × (0.5–1% solids)[c]
Mascara (195)	1	—	—
Other Eye Makeup Preparations (152)	5	1	Not available
		0.1–5	1 × (0.5% solids)[c]
Colognes and Toilet Waters (684)	1	1	Not available
		1	100 (5% solids)[c]
Powders (Dusting and Talcum) (273)	3	0.0005	200 × (92% solids)
Other Fragrance Preparations (173)	9	20	0.5
Hair Conditioners (651)	50	2	0.9
		0.01–1	100
Hair Sprays (Aerosol Fixatives) (275)	4	0.0007	0.9
		0.0001	1×[c]

TABLE 8
Product formulation data for aloe-derived ingredients *(Continued)*

Product category (number of formulations in each category) (FDA 2002)	Formulations containing ingredient (FDA 2002)	Concentration of raw material in product (%)[a] (CTFA 2002)	Concentration of Aloe in raw material (% solids) (CTFA 2002)
Hair Straighteners (63)	1	0.0001	0.5
Permanent Waves (207)	4	—	—
Rinses (Noncoloring) (42)	2	—	—
Shampoos (Noncoloring) (884)	47	0.01–0.05	Not available
		2	0.9
		0.01	100
Hair Tonics, Dressings, etc. (598)	26	0.1	0.9
Wave Sets (53)	4	—	—
Other Hair Preparations (277)	2	0.0002	0.9
Hair Dyes and Colors (1690)	21	0.1	5
Hair Color Sprays (Aerosol) (5)	1	—	—
Hair Bleaches (120)	7	—	—
Face Powders (305)	1	0.1	Not available
		0.1	1 × (0.5% solids)[c]
Foundations (324)	3	0.5–4	1 × (0.5% solids)[c]
Lipstick (962)	—	0.5	92
Makeup Bases (141)	1	—	—
Rouges	—	0.05	1 × (0.5% solids)[c]
Other Makeup Preparations (201)	3	0.2	1 × (0.5% solids)[c]
Cuticle Softeners (19)	1	—	—
Nail Creams and Lotions (15)	—	0.01–2	1 × (2–5% solids)[c]
Other Manicuring Preparations (55)	3	—	—
Mouthwashes and Breath Fresheners (46)	1	—	—
Bath Soaps and Detergents (421)	15	0.5	5
		0.0001	0.1
		0.05–1	1 × (0.5–1% solids)[c]
		0.01	200 × (∼90% solids)[c]
Deodorants (Underarm) (247)	5	—	—
Douches (5)	—	1	1 × (0.5% solids)[c]
Feminine Hygiene Deodorant (4)	—	0.1	Not available
Other Personal Cleanliness Products (308)	29	1–2	1 × (0.5–5% solids)[c]
Aftershave Lotions (231)	8	20	Not available
		0.5	5
		0.1	92
		1–20	1 × (0.5–2% solids)[c]
Shaving Cream (134)	10	0.5	Not available
		0.1–0.5	100
Other Shaving Preparation Products (63)	9	1–2	1 × (0.5–5% solids)[c]
Skin Cleansing Creams, Lotions, Liquids (775)	22	0.5	5
		0.01–0.5	0.1–5
		0.5–7	1 × (0.5–5% solids)[c]
Depilatories (34)	3	—	—
Face and Neck Skin Preparations (310)	14	0.5	5
		0.1–0.5	0.1–1
		0.05–2	1 × (0.5–1% solids)[c]

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TABLE 8
Product formulation data for aloe-derived ingredients *(Continued)*

Product category (number of formulations in each category) (FDA 2002)	Formulations containing ingredient (FDA 2002)	Concentration of raw material in product (%)[a] (CTFA 2002)	Concentration of Aloe in raw material (% solids) (CTFA 2002)
Body and Hand Skin Care Preparations (840)	38	0.5	5
		0.2	92
		3	0.9
		1–20	1 × (0.5–1% solids)[c]
		0.01	200 × (~90% solids)[c]
Body and Hand Sprays (35)	—	0.001	0.1
Foot Powders and Sprays (35)	—	0.1	100 (5% solids)[c]
Moisturizers (905)	59	0.5	5
		1–3	1 × (0.5–5% solids)[c]
Night Creams, Lotions and Powders (200)	3	0.5	92
		1–5	0.5–1
Paste Masks (Mud Packs) (271)	8	1	Not available
		5	0.5
Skin Fresheners (184)	14	0.1–2	1 × (0.5–1% solids)[c]
		0.03	5
Other Skin Care Preparations (725)	32	2	Not available
		0.01	92
		0.1–3	1 × (0.5–5% solids)[c]
		0.1	40 × (21% solids)[c]
Suntan Gels, Creams, and Liquids (131)	8	3–4	1 × (0.5% solids)[c]
		0.1	40 × (21% solids)[c]
Indoor Tanning Preparations (71)	9	0.02	92
Other Suntan Preparations (38)	3	5	1 × (0.5% solids)[c]
Total	529	0.0001–20	
	Aloe, Powdered[b]		
Bath oils, tablets, and salts (143)	—	0.1	100 (powder)
Eye Lotion (25)	—	0.05	100 (powder)
Other Eye Makeup Preparations (152)	—	0.01	100 (powder)
Fragrance Powders (273)	—	0.1	100 (powder)
Blushers (All Types) (245)	2	0.1	100 (powder)
Face Powders (305)	—	0.05	100 (powder)
Foundations	—	0.01	100 (powder)
Bath Soaps and Detergents (421)	1	—	—
Other Personal Cleanliness Products (308)	—	0.01	100 (powder)
Body and Hand Skin Care Preparations (840)	1	0.0003	200× (powder)
Moisturizers (905)	—	0.2	100 (powder)
Other Skin Care Preparations (63)	—	0.00001	200× (powder)
	—	0.01	100 (powder)
	—	0.1	100 (powder)
Other Suntan Preparations (38)	—	0.0003	200× (powder)
Total	4	0.00001–0.2	
	Aloe Ferox Leaf Extract[b]		
Colognes and Toilet Water (684)	—	5	0.002
Rinses (Noncoloring) (42)	—	0.1	0.002
Shampoos (Noncoloring) (884)	—	0.1	0.002

TABLE 8
Product formulation data for aloe-derived ingredients *(Continued)*

Product category (number of formulations in each category) (FDA 2002)	Formulations containing ingredient (FDA 2002)	Concentration of raw material in product (%)[a] (CTFA 2002)	Concentration of Aloe in raw material (% solids) (CTFA 2002)
Bath Soaps and Detergents (421)	—	0.1	0.002
Other Personal Cleanliness Products (308)	—	0.1	0.002
Aftershave Lotions (231)	—	0.08	2.5
Body and Hand Skin Care Preparations (840)	—	5	0.002
Body and Hand Sprays (35)	—	5	0.002
Moisturizers (905)	—	0.001	3
		0.1	0.6
Other Skin Care Preparations	—	0.1	0.002
Total	None	0.001–5	

[a]In a given product category, there may be more than one industry report of a concentration of raw material in such products. All data provided are given, but the number of products at each concentration is unknown.
[b]Reports to FDA did not correspond to current ingredient terminology. See text.
[C]As reported.

Aloe-derived materials, not specified, are used as drug products containing certain active ingredients offered over the counter as orally administered menstrual drug products (21 CFR 310.545). Aloin, an active ingredient in Aloes, is used as a drug product containing certain active ingredients offered over the counter as a stimulant laxative (21 CFR 310.545). Aloe extract and aloe flower extract used in over-the-counter (OTC) drug products as a stimulant laxative were not recognized and are not generally recognized as safe (GRAS) and effective or are misbranded due to a lack of carcinogenicity data (21 CFR 310.525(a)12(iv)(C)).

Sturm and Hayes (1984) stated that Aloe may be used in medicated liners in immediate dentures, and as an anti-inflammatory agent after brushing or oral surgery, in addition to mouth rinses and toothpastes.

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Hirata et al. (1981) administered 10 mg ^{14}C-labeled aloenin (see Figure 1), derived from *Aloe arborescens*, in water orally to 2-month-old rats after an overnight fast. The feces and urine were collected 24 and 48 h after administration. Methanol (MeOH) extraction of the feces and urine indicated that most of the ^{14}C-labeled aloenin was excreted within the first 24 h. Only a small amount was excreted within the next 24-h period. Thin-layer chromatography indicated that aloenin was metabolized to 4-methoxy-6-(2,4-dihydroxy-6-methylphenyl)-2-pyrone, 2,5-dimethyl-7-hydroxychromone, and glucose. The distribution and/or accumulation of aloenin in the stomach, liver, and kidneys indicated that aloenin and its metabolites accumulated in the liver and the kidneys.

Ishii et al. (1987) administered barbaloin (see Figure 1), dissolved in distilled water at 20 mg/ml, orally to male Wistar Rats at a dose of 100 mg/kg. At specific time points after administration, blood was withdrawn from the rats to measure the serum levels of barbaloin. Barbaloin was first seen in the serum 30 min (0.092 μg/ml) after administration with maximum concentration (0.337 μg/ml) occurring at 90 min. After the 90-min time point, concentrations of barbaloin decreased smoothly with detection still possible at 6 h after administration.

Barbaloin (31.1 mg/5 ml/kg in 5% gum arabic solution) administered by cecal intubation to male Wistar rats (150 to 200 g), produced aloe-emodin-9-anthrone 1 h after administration. Aloe-emodin-9-anthrone, a decomposition product of barbaloin, was measured by thin-layer chromatography. Aloe-emodin-9-anthrone peaked after 4 h of administration of the barbaloin and was found in the cecum at levels of 508 μg/rat and in the colon at 83 μg/rat (Ishii et al. 1994).

Lang (1993) administered aloe-emodin (see Figure 1) to SPF Brown-Norway rats (numbers not given) orally in a tragacanth (0.3%) suspension at a dose of 4.5 mg/kg. The aloe-emodin had been previously labeled with ^{14}C. Blood, feces, urine, and 24 organs were collected at specific time points to elucidate the distribution of aloe-emodin. Maximum blood concentrations were reached 1.5 to 3 h post administration and were 248 ng (males) and 441 ng (females) equivalents aloe-emodin/ml. During the first 6 h post administration there were no differences in organ concentrations between the males and females; however, in later

samples females had higher concentrations than the males. The liver and kidney were the only organs that had higher concentrations of aloe-emodin than plasma. Three-fourths of the dose was excreted in the feces within the first 2 days. Plasma protein binding of aloe-emodin was determined in vitro and ex vivo. The results were an in vitro binding of 98% to 99% and an ex vivo binding of 86% to 96%. No sex differences were found.

Heidemann et al. (1996) reported that NMRI and DBA mice treated with a single oral dose (2000 mg/kg) of aloe-emodin had blood plasma concentrations ranging from 3 to10 μg/ml. NMRI mice and Wistar rats treated with a single oral dose of aloe-emodin (1500 or 2000 mg/kg) had maximum blood plasma concentrations of 17 μg/ml after 3 h.

Yagi et al. (1999) administered aloemannan (a polysaccharide of *Aloe barbadensis*) to mice at 120 mg/kg orally or by intravenous injection (number and strain of mice were not given). Prior to administration, the aloemannan had been labeled with fluorescein isothiocyanate (FITC-aloemannan). Examination of the urine and feces showed the FITC-aloemannan was metabolized into smaller molecules that accumulated in the kidneys. Intravenous administration resulted in a greater quantity of FITC-aloemannan in the urine within the first 24 h, with minimal amounts found in the feces over the 48-h period. Oral administration resulted in a greater quantity of FITC-aloemannan in the feces within the first 24-h period when compared to the urine.

Fungicidal Activity

Fujita et al. (1978) tested a whole leaf powder of *Aloe arborescens* and an *Aloe arborescens* leaf homogenate (high-molecular-weight) lyophilized powder against three strains of *Trichophyton mentagrophytes*. Both powders were shown to have fungicidal activity against *T. mentagrophytes*. The minimum inhibitory concentration (MIC) was 25 mg/ml for the whole-leaf powder and 10 mg/ml for the high-molecular-weight powder. Both the whole-leaf powder and the high-molecular-weight component powder induced various morphological abnormalities in spores and hyphae by the inhibition of spore germination and development of hyphae.

Ali et al. (1999) screened extracts of fresh leaves of *Aloe arborescens* and *Aloe barbadensis* for their antifungal activity against *Aspergillus niger, Cladosporium herbarum*, and *Fusarium moniliforme*. The solvent used for the extraction affected fungicidal activity. Ethanol extraction was the most effective followed by chloroform, benzene, and water. All extracts of *Aloe arborescens* and *Aloe barbadensis* had some fungicidal activity.

Antimicrobial Activity

Lorenzetti et al. (1964) made a preparation of Aloe vera leaves by cutting them at the base and standing them upright to allow the juice to drain out. This juice was heated for 15 min at 80°C and then freeze dried. The freeze-dried juice was reconstituted in distilled water (20 mg/ml) and tested in the agar diffusion test against: *Staphylococcus aureus 209, Escherichia coli, Streptococcus pyogenes, Corynebacterium xerose, Shigella paradysenteriae, Salmonella typhosa, Salmonella schottmuelleri*, and *Salmonella paratyphi*. Significant inhibition of growth occurred with *S. aureus 209, S. pyogenes, C. xerose, and S. paratyphi*.

Northway (1975) used an *Aloe vera* product, in three forms, topically to treat a variety of infections in 76 animals. The Aloe products were 100% *Aloe vera* gel (Aloe 99 Gel), 75% *Aloe vera* gel in a cream (Aloe 99 Creme), and 82% *Aloe vera* gel in a lotion (Aloe 99 Lotion). The active ingredient was derived from the mature leaves of 4-year-old *Aloe vera* plants. Treatment was from 1 to 4 weeks depending on condition. Except in the cases of abscesses, treatment consisted of thoroughly rubbing the medication into the lesion two to four times daily. Abscesses were infused with medication twice daily. In otitis externa, the ear was cleansed once a day and the medication applied and worked into the canal twice daily. The medication was worked into the staphyloma four times daily for a month. Results are summarized in Table 9.

The author concluded that the aloe products appeared to retard exuberant granulation tissue and that pain and itching were relieved promptly. No toxic reactions or other adverse effects were seen in any animal (Northway 1975).

Heggers et al. (1979) tested *Aloe vera* gel and Dermaide Aloe (a commercially prepared purified extract) against 10 bacterial strains. Sixty percent to 90% concentrations were used to inoculate *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Escherichia coli, Serratia marcescens, Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., *Bacillus subtilis,* and *Candida albicans*. At 90%, *Aloe vera* gel was effective against all organisms, but at the 70% concentration only *S. pyogenes* was inhibited. Dermaide Aloe was effective against all organisms at a concentration of 70%.

Heck et al. (1981) tested a preserved Aloe gel extract (from a commercial provider) and an unpreserved Aloe extract that resembled the one that could be obtained from a household plant against *Pseudomonas aeruginosa, Enterobacter aerogenes, Staphylococcus aureus,* and *Klebsiella pneumoniae*. Concentrations of each of the extracts (10%, 20%, 40%, 70%, 80%, 90%) were inoculated by 10^2 and 10^6 concentrations of each bacterium. The preserved Aloe gel extract was effective in controlling bacterial growth at a concentration of 40%: none of the cultures of *Pseudomonas, Enterobacter,* or *Klebsiella* had any growth, *Staphylococcus* had growth in two out of nine cultures. Only the 90% concentration of the unpreserved Aloe extract had any effect on the bacterial cultures and even that was not consistent.

Robson et al. (1982) studied the antibacterial effects of *Aloe vera* extract and found that concentrations as low as 60% were bactericidal against 7 of the 12 species of organisms studied. These were *Citrobacter* sp., *Serratia marcescens, Enterobacter cloacae, Klebsiella pneumoniae, Pseudmonas aeruginosa, Streptococcus pyogene,* and *Streptococcus agalactiae*. Concentrations between 80% and 90% were bactericidal for the above

TABLE 9
Aloe vera treatment for various conditions (Northway 1975)

Condition treated	Number of animals	Species			Response*			
		Dog	Cat	Other	E	G	P	N
Ringworm**	14	4	10	—	4	10	—	—
Atopy (allergy)	12	10	2	—	6	6	—	—
Abscess	12	1	11	—	—	12	—	—
Otitis externa	11	8	3	—	—	9	—	2
Hot spots	11	9	2	—	—	10	1	—
Misc. fungal infections	9	7	1	—	2	7	—	—
Lacerations***	4	—	—	1 (rodent)	—	4	—	—
Lip fold dermatitis	1	1	—	4 (horses)	—	—	1	—
Inflamed cyst	1	1	—	—	—	1	—	—
Staphyloma	1	1	—	—	—	1	—	—

*E = Excellent (better than other drugs on market); G = Good (equal to the best drugs on market); P = Poor (not as good as other drugs on market); N = No response.

**Fluorescent under ultraviolet light.

***Exuberant granulation tissue removed surgically in two horses, then *Aloe vera* Gel applied.

species and the other species of the organisms studied, viz. *Staphylococcus aureus, Escherichia coli, Streptococcus feacalis, Bacillus subtilis,* and *Candida albicans* (yeast).

Antiviral Activity

Kahlon et al. (1991a) tested acemannan (concentrations ranged from 3.2 to 1000 μg/ml) in a variety of cell lines (human peripheral blood mononuclear cells [PBMCs], CEM-SS and MT-2) for antiviral activity against human immunodeficiency virus [HIV]-1. Maximum inhibitory effect was observed in CEM-SS cells (infected with HIV-1); it was concentration dependent. Fifty percent inhibition occurred at concentrations of 45 to 48 μg/ml, whereas 100% inhibition occurred at 1000 μg/ml; at this second concentration no cytotoxic effects were observed. Acemannan, at a concentration of 31.25 μg/ml, suppresses viral-induced syncytia formation, with inhibition occurring at 62.5 μg/ml. In PBMCs, acemannan caused a concentration dependent inhibition of HIV-1 replication. Maximum suppression occurred at concentrations of 62 or 125 μg/ml.

Kahlon et al. (1991b) tested acemannan, at various concentrations, alone or in conjunction with either azidothymidine (AZT) or acyclovir. Acemannan (concentrations ranged from 15.6 to 250 μg/ml), with or without AZT, was tested in vitro in mature human T4 lymphocytes infected with HIV-1. Acemannan, at a concentration of 125 μg/ml, inhibited replication of HIV-1 cells; however, 15.62 μg/ml of Acemannan and 0.001 or 0.01 ng/ml of AZT produced 96% and 100% inhibition, respectively. Acemannan (0 to 100 μg/ml concentration) was tested with or without acyclovir, in vitro, in herpes simplex virus (HSV)-1–infected Vero cells. Acemannan alone was not able to significantly reduce replication of HSV-1–infected cells; however Acemannan (40 μg/ml) combined with 0.025 μg/ml of acyclovir inhibited replication by $\geq$90%.

Sydiskis et al. (1991) tested hot glycerin extracts of *Aloe barbadensis* against a variety of viruses, including herpes simplex virus (HSV-1 and HSV-2), pseudorabies virus (PSV), varicella-zoster virus (VZV), influenza virus (INF), rhinovirus (RH), and adenovirus (AD). The active hot glycerin extract of *Aloe barbadensis* was determined to contain aloe-emodin, so that compound was tested in the same assays. The hot glycerin extracts of *Aloe barbadensis* were virucidal to HSV-1 within 15 min of incubation. The effect of the hot glycerin *Aloe barbadensis* extract on HSV-1 inactivation was concentration dependent and occurred more rapidly at 37°C than 4°C. Aloe-emodin at a concentration of 0.1 mg/ml in 50% glycerin was active against HSV-1, HSV-2, pseudorabies virus, varicella-zoster virus, and influenza virus. However, at the highest concentration tested, rhinovirus and adenovirus were not affected when compared to the controls (50% glycerin). Aloe-emodin–treated Vero or WI-38 cells did not exhibit altered morphology, indicative of cytotoxicity, when compared to the controls.

Cytotoxicity

Brasher et al. (1969) incorporated solutions of prednisolone, indomethacin, and *Aloe vera* gel into cell maintenance medium to look for cytotoxicity in HeLa cells and rabbit kidney fibroblasts. *Aloe vera* gel at the 5×10^{-1} dilution (no concentration units given) was toxic to both cell lines at all hours examined. Aloe vera gel that was diluted 10^{-1}, 10^{-2}, and 10^{-3} did not have any significant effect on the cell lines.

Winters et al. (1981) conducted a study in which fresh leaves from *Aloe barbadensis* were minced, homogenized, and centrifuged to give two different fractions, the supernatant and the

pellet. Aloe vera gel was also purchased off the shelf from a local store and underwent the same procedure. The supernatant and the pellet from both sources (concentrations were not given) were used in four different assays (hemagglutination titration, immunodiffusion, cell attachment and growth, and wounded cell monolayer) to evaluate cytotoxicity. The supernatant fraction of the fresh leaves and the commercial preparation were found to have high levels of lectin-like substances as seen with the hemagglutination titration and immunodiffusion assays. The supernatant fraction from *Aloe barbadensis* was found to markedly promote cellular attachment and growth of normal human cells but not cancer cells; however, the commercial *Aloe vera* gel (fractions not specified) was cytotoxic for human normal and tumor cells in vitro. Additionally, the fractions from *Aloe barbadensis* enhanced the healing of wounded cell monolayers.

Danof and McAnalley (1983) tested four commercial preparations (description not given) of stabilized Aloe vera gel samples for their cytotoxicity in human endothelial cells and fibroblasts. In addition, the yellow sap from fresh *Aloe vera* was tested. The yellow sap at all concentrations was lethal to human fibroblasts. Two of the four products were cytotoxic to both cell types; one showed significant toxicity.

Bouthet et al. (1995) studied the effect of aloe on cultures of human lung embryonic (HEL) cells and rat adrenal pheochromocytoma (PC12) cells. Liquid extract of whole leaf Aloe (*Aloe barbadensis*) and the gel filet portion of Aloe leaves (both purchased commercially) were lyophilized and then reconstituted with sterile water. Both the liquid extract of whole leaf Aloe and the gel filet portion of Aloe were added to cell cultures to measure their effects on cell proliferation. Control cells received an equal amount of media. The liquid extract of whole leaf Aloe significantly stimulated the growth of the PC12 cells at concentrations of 0.162 and 0.312 μg/ml. The liquid extract of whole leaf Aloe stimulated PC12 cell growth quicker and better than the HEL cells. The gel filet portion of Aloe (0.162 and 0.625 μg/ml) significantly stimulated the growth of OC12 cells after 2 days. The gel filet portion of Aloe had little or no effect on HEL cell proliferation. Differentiation of PC12 cells did not occur with either the liquid extract of whole leaf Aloe or gel filet portion of Aloe.

Avila et al. (1997) isolated three fractions from the leaves of *Aloe barbadensis*: native gel (mucilaginous parenchymous tissue scraped from Aloe leaves), purified gel (removal of debris), and a low-molecular-weight fraction (LMWF). The authors used the three fractions, along with purchased aloin, to evaluate the cytotoxicity of the LMWF (50 μg/ml). The cell injury assay was performed with fibroblasts that were cultured from chicken eggs. A 1:10 dilution of native gel (diluted with Dulbecco's minimal essential medium) caused cell injury, whereas the purified gel did not cause injury when tested at the same dilution. The LMWF behaved as the native gel did. Aloin also damaged the cells.

Tello et al. (1998) cultured commercially available human fibroblasts with formulations of acemannan in preservative (ratios ranged from 20:1 to 150:1). Details of the formulations were not given. A 1% concentration of the solutions was incubated for 3, 6, 12, and 24 h. Formulations of acemannan had significant cytotoxicity, especially in the formulations with a high level of preservative, although one formulation had minimal cytotoxicity.

Enzyme Activity

Yagi et al. (1985) isolated a 40,000 molecular weight (MW) glycoprotein from *Aloe arborescens* that stimulated DNA synthesis of BHK-21 cells at 5 μg/ml. The cells did not have any morphological changes.

Yagi et al. (1987), repeating the work of Fujita et al. (1976), reported that an Aloe glycoprotein isolated from *Aloe arborescens* appeared to have enzymatic activity against bradykinin. Extracts of fresh leaves of *Aloe arborescens* were homogenized and centrifuged to isolate a glycoprotein of 40,000 MW. The glycoprotein (1 mg) was incubated with 10 μg of bradykinin for 10 min. The bradykinin solution (0.2 ml) was incubated with isolated guinea pig ileum for 5 min. The contractile response of the ileum was measured. The Aloe glycoprotein degrades bradykinin.

Norton et al. (1990) examined the outer green rind of fresh *Aloe barbadensis* leaves for possible glyoxalase activity. Purification was done by affinity ligand-enzyme binding. Glyoxalase I, a basic protein with a molecular weight of 44,000, and glyoxalase II, an acidic protein with a molecular weight of 27,000, were isolated.

Sabeh et al. (1993) isolated glutathione peroxidase from the inner mucilaginous parenchymal tissue of *Aloe barbadensis*. The enzyme has a molecular weight of 62 kDa and is composed of four identical subunits. Sabeh et al. (1996) identified seven superoxide dismutases in the inner mucilaginous parenchymal tissue (produces the gel).

Lee et al. (1997) reported that aloesin has cell-growth stimulatory activity. DNA synthesis of SK-HEP-1 cells treated with aloesin was stimulated in a dose-dependent manner. At the 1 to 50 μM concentration range, aloesin stimulated DNA synthetic activity two- to fourfold over controls. The authors further determined that aloesin significantly increased intracellular levels of cyclin E, CDK2, and CDC 25A in SK-HEP-1 cells.

Yagi et al. (1997) prepared two fractions from the leaf gel of *Aloe barbadensis* by column chromatography; one fraction was a 29-kDa glycoprotein (later named verectin) and the other was a neutral polysaccharide. Both fractions were then tested with either BHK-21 or normal human dermal cells. The glycoprotein fraction promoted cell growth; however, the neutral polysaccharide fraction did not stimulate cell growth.

Esteban et al. (2000) tested commercial *Aloe vera* gel preparations and the inner aqueous leaf parenchyma of *Aloe barbadensis* for possible peroxidase activity. Peroxidase activity was detected in the *Aloe barbadensis* preparation and the commercial preparation. Both preparations lost their peroxidase activity when heated

for 5 min in a water bath at 100°C. The peroxidase activity of the commercial preparations varied.

Immunological Effects

t'Hart et al. (1988) excised mucilaginous parenchymal tissue from fresh *Aloe vera* leaves, lyophilized, reconstituted with Hanks' balanced salt saline (HBSS) and centrifuged to obtain a high-molecular-weight and a low-molecular-weight fraction. Each fraction was tested with pooled human serum for complement activity. The high-molecular-weight fraction depleted the classical and alternative complement systems. The low-molecular-weight fraction inhibited the production of oxygen free radicals by polymorphonuclear leukocytes.

Marshall et al. (1993) incubated acemannan, in concentrations ranging from 0 to 100 μg/ml, overnight with human peripheral blood mononuclear cells. Cytokine concentrations were assayed by enzyme-linked immunosorbent assay (ELISA). Acemannan stimulates tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6.

Strickland et al. (1994) investigated the ability of *Aloe barbadensis* gel extract to prevent the suppression of contact hypersensitivity (CHS) and delayed-type hypersensitivity (DTH) responses in mice by ultraviolet (UV) radiation. Treatment groups contained five C3H/Hen(MTV$^-$) female mice. The mice in the local suppression group received four daily doses of 400 J/m^2 of UVB on their abdomens. The systemic suppression group received either a single dorsal exposure of 5 kJ/m^2 (DTH suppression) or 10 kJ/m^2 (CHS suppression). Control mice did not receive UVB irradiation. Irradiated mice were treated with the *Aloe barbadensis* gel extract (in Aquaphor) (0.167%, 0.5%, or 1.67%) within 5 min after irradiation. Approximately 75 mg of vehicle control (Aquaphor) or *Aloe barbadensis* gel extract in vehicle was applied to each mouse. CHS was induced by a solution of 0.5% fluorescein isothiocyanate (FITC) and DTH was induced with formalin-fixed *Candida albicans*. Treatment of UV-irradiated skin with the *Aloe barbadensis* gel extract (all concentrations) prevented the induction of local and systemic suppression of both CHS and DTH. The *Aloe barbadensis* gel extract treatment partially preserved the number and morphology of Langerhans and Thy-1$^+$ dendritic epidermal cells in skin, compared to those in the skin of mice given only UV radiation or UV radiation plus the vehicle control.

Tyler (1994) postulated that some of the beneficial effects of aloe are the result of a carboxypeptidase that inhibits the pain-producing agent bradykinin. The enzyme is also believed to hinder the formation of thromboxane, the activity of which is detrimental to burn wound healing. Antiprostaglandin activity was also suggested.

Cultures of normal chicken spleen cells and HD11 line cells produce nitric oxide in response to Acemannan extracted from *Aloe vera* plant (Karaca et al. 1995). The nitric oxide inducing effect of Acemannan on spleen cells was dose dependent. Acemannan-induced nitric oxide synthesis may be mediated through macrophage mannose receptors, and macrophage activation may be accountable for some of the immunomodulatory effects of Acemannan in chickens. Macrophage activation may also account for some of the wound healing capabilities attributed to Acemannan.

Lee et al. (1995) tested dichloromethane extracted *Aloe vera* gel (yielded two low-molecular-weight fractions) for angiogenic activity in the chick embryo chorioallantoic membrane assay. Both fractions (100 and 250 μg/egg) produced a significant angiogenic effect. The effect was similar to phorbol-12-myristate-13-acetate (PMA) (positive control).

Egger et al. (1996) injected CARN 750 (dissolved in distilled phosphate-buffered saline [D-PBS]), isolated from *Aloe barbadensis*, subcutaneously into female C_{57}BL/6 mice (number not given) at doses of 0.05, 1, or 2 mg/animal for 18 days. Negative-control animals received PBS on a similar schedule and positive-control animals received 3 μg of granulocyte colony-stimulating factor (G-CSF). Bone marrow and spleen cells were obtained from the euthanized animals. The administration of CARN 750 significantly increased splenic and peripheral blood cellularity as well as hematopoietic progenitors in the spleen and bone marrow. In myelosuppressed mice, CARN 750 had activity equal to or greater than G-CSF.

Zhang and Tizard (1996) tested acemannan, from the central gel of the leaf of *Aloe barbadensis*, for its effects on the mouse macrophage cell line RAW 264.7. The cells were treated with either acemannan alone (100 μg/ml), interferon (IFN)-γ alone (10 U/ml; positive control), or acemannan/IFN-γ (acemannan 100 μg/ml/ IFN-γ 10 U/ml) for 24 h. Acemannan stimulated macrophage cytokine production, nitric oxide release, surface molecule expression, and cell morphologic changes. The production of the cytokines IL-6 and TNF-α were dependent on the dose of acemannan.

Lee et al. (1997) prepared epidermal cells from mouse ear skin and exposed them, in vitro, to a single dose of 180 J/m^2 of UVB radiation. The epidermal cells were used to determine what fraction of *Aloe vera* gel would be able to prevent UV-B–induced impairment of Langerhans cells. Lyophilized *Aloe vera* gel (provided by the Aloe Research Foundation) was dissolved in distilled water and then separated into four fractions based on molecular size: <500, between 500 and 1000, between 1000 and 3000, and >3000. After testing the four fractions with the mouse epidermal cells, the fraction that was found to be in the 500 to 1000 MW range was further fractionated by a Bio-Gel P-2 column into five fractions: G1C2F0, G1C2F1, G1C2F2, G1C2F3, and G1C2F4. The fraction G1C2F1 (low-molecular-weight fraction) gave the strongest response in preventing UV-B–induced impairment of Langerhans cell function.

In the course of their investigation of *Aloe barbadensis* gel's ability to restore CHS and DTH that had been suppressed by UV radiation, Byeon et al. (1998) discovered that the responses varied between lots. Four lots were obtained from the Aloe Research Foundation (manufactured by Aloecorp, Harlington, TX): ARF'91, ARF'94B, ARF'94G, and ARF'94K. Three

months after its manufacture, lot ARF'91G (this lot was not mentioned previously in the methods and materials section) was only able to restore 43% of the CHS response, whereas lot ARF'91A completely restored the CHS response. After 9 months of storage as a lyophilized powder, none of the extracts from the lots prevented UV-induced suppression of CHS. It was also discovered that commercially prepared *Aloe barbadensis* gels were uniformly inactive even when tested within 1 month of their manufacture.

In contrast, all lots of *Aloe barbadensis* gel prevented systemic suppression of DTH. The immunoprotective activity remained unchanged after 12 months of storage. The different decay rates observed in the CHS and DTH protective activity indicated that Aloe gel contains multiple factors that act on the two different models of immune suppression. The labile of the factor that prevents suppression of CHS responses varies in different lots of the specifically prepared gel extract and was influenced by the manufacturing process used to produce the extracts. The finding that biologically active components in Aloe can be labile helps to explain some of the difficulties that investigators have reported in reproducing results using unfractionated materials from the plant (Byeon et al. 1998).

Ramamoorthy and Tizard (1998) treated raw 264.7 cells (a mouse monocytic-macrophage cell line) with acemannan (50 μg/ml), IFN-γ (1 unit/ml), or a combination of acemannan and IFN-γ to investigate acemannan's ability to induce apoptosis. After 18 h of treatment with acemannan, apoptosis was evident, and by 36 and 48 h, 65% and 100%, respectively, of the cells had undergone apoptosis.

Lee et al. (1999) exposed pathogen-free male C57BL/6 mice (number not given) to UVB radiation. The dose used (2.4 kJ/m^2) was the optimal dose needed to suppress contact hypersensitivity (CHS). The irradiated skin was immediately treated with G1C2F1 cream (isolated component of *Aloe vera* mixed with polyethylene glycol to form a cream). A single dose of 0.1 ml/mouse reduced contact hypersensitivity suppression significantly. The percentage recovery of UVB–suppressed CHS response was 52.3%, 77.3%, and 86.6% when the irradiated skin was treated once with 0.1, 0.5, and 2.5 mg/ml of G1C2F1 cream, respectively. Topical application of G1C2F1 prevented UVB–induced suppression of contact hypersensitivity response by possible restoration of the immune response.

Strickland et al. (1999) exposed pathogen-free female C3H/HeN and BALB/C mice (5 animals/group) to 5 to 30 kJ/m^{2}dose of UVB radiation. *Aloe barbadensis* poly/oligosaccharides in phosphate-buffered saline or control polysaccharides (methylcellulose or dextran) were applied immediately after UVB exposure. The poly/oligosaccharides of *Aloe barbadensis* prevented the UV-B–induced suppression of the DTH response whereas the control polysaccharides did not. Additionally, Pam212 keratinocytes were exposed to 300 J/m^2 UVB radiation and then treated with *Aloe barbadensis* poly/oligosaccharide. Control cells were similarly treated but not exposed to the UVB radiation. The poly/oligosaccharide of *Aloe barbadensis* decreased (50%) IL-10 production.

Qui et al. (2000) tested a modified aloe gel extract (MAP) (MW 80 Da) from *Aloe barbadensis* for its immunomodulatory activity. Peritoneal macrophages isolated from ICR mice were incubated overnight with MAP, native Aloe gel, or lipopolysaccharide (LPS). MAP stimulated TNF-α release from the macrophages in a dose-dependent manner, with the greatest release of TNF-α occurring at a concentration of 250 μg/ml. The LPS data mimicked the MAP data. The native Aloe gel was unable to stimulate TNF-α release from macrophages. The MAP was also tested for its ability to prevent UVB irradiation–induced immune suppression. SPF female C3H/HeN mice were exposed to 2000 J/m^2 of UVB; control animals did not receive irradiation. Immediately after exposure, the mice were either dosed with the vehicle (water in Aquaphor) or MAP in Aquaphor. The mice were sensitized on their abdomens 3 days later with 0.3% dinitrofluorobenzene (DNFB), with challenge on the ears with 0.2% DNFB occurring 6 days after the sensitization. In the control groups, the vehicle and MAP did not affect the challenge; however, MAP had, in a dose-dependent manner, UV-B–protective contact hypersensitivity activity.

Lee et al. (2001) reported that acemannan induced maturation of dendritic cells (DCs) isolated from C57BL/6 mouse bone marrow in vitro. Phenotypic maturation was evident with the up-regulation of class II major histocombatibility complex (MHC) molecules and costimulatory molecules such as B7-1, B7-2, CD40, and CD54. There was also increased allogenic T-cell stimulatory activity and production of cytokine IL-12. The DC maturation was dose dependent (0, 10, 100 μg/ml acemannan). The higher exposure group had a more potent allogenic T-cell stimulatory activity compared to the low exposure. Mannose (acemannan is a polymer of acetylated mannose) did not have a significant effect on the acemannan-induced phenotypic and functional maturation of immature DCs. However, mannose suppressed the mitogenic activity.

Pugh et al. (2001) performed a macrophage activation assay on aloeride (a polysaccharide) to determine if this fraction from *Aloe barbadensis* was the fraction responsible for the immunostimulatory properties that have been attributed to *Aloe vera*. Aloeride activated the transcription factor nuclear factor (NF)-κB at a concentration of 200 μg/ml.

HYPOGLYCEMIC/HYPERGLYCEMIC ACTIVITY

Dixit and Joshi (1983) studied the effect of an aqueous extract of *Aloe barbadensis* in Triton WR–induced hyperlipidemia in the Presbytis monkey. Fresh leaves of *Aloe barbadensis* were ground at room temperature and the yellow mucoid fluid was decanted, dried, and used for this study. Ten adult male *Presbytis entellus* monkeys (11 to 16 kg) were deprived of food for 12 to 16 h and then injected with Triton WR (200 mg/kg body weight) to induce a hyperlipidemic state. After the monkeys were confirmed to be hyperlipidemic, they were orally

administered 200 mg/kg body weight of the aqueous extract of *Aloe barbadensis* or clofibrate, a comparative control drug (five monkeys per group). Blood was withdrawn 4, 8, 12, and 24 h after dose administration. Immediately after the 24-h blood sampling, a second dose of the aqueous extract of *Aloe barbadensis* and clofibrate (same dose) were administered. Blood was withdrawn 4, 8, 12, and 24 h after the second dose. A significant decrease of total cholesterol was observed 28 h after drug administration.

The aqueous extract of *Aloe barbadensis* decreased the total cholesterol level by 61.6%, triglyceride level by 37.8%, phospholipid level 51.2%, and nonesterified free fatty acids by 45.5%. In comparison, clofibrate decreased the total cholesterol level by 47.6%, triglyceride by 50%, phospholipid by 41.7%, and 23% for nonesterified free fatty acids. A significant decrease of low-density lipoprotein (LDL) cholesterol (79%) and very-low-density lipoprotein (VLDL) cholesterol (37.5%) occurred in the monkeys treated with the aqueous extract of *Aloe barbadensis* as compared to the control monkeys. The high-density lipoprotein (HDL)/total cholesterol ratio in the aqueous extract of *Aloe barbadensis*–dosed monkeys was significantly increased at all intervals compared to their initial values (Dixit and Joshi 1983).

Ghannam et al. (1986) studied two groups of five fasting normal Swiss albino mice to determine the effect of the solid residue obtained after evaporating the sap of various species of aloe on fasting plasma glucose (FPG) level. The negative-control group received a single oral dose of normal saline. The test group received an oral aqueous suspension of Aloes, 500 mg/kg. The aqueous suspension consisted of the dried sap of Aloes purchased from a local market. Plasma glucose was measured at 0 (before treatment), 2, 4, and 6 h after treatment. A single oral dose of the Aloes failed to lower the FPG levels of the mice after 2, 4, or 6 h after treatment. No adverse effects were reported.

These authors also orally dosed 25 normal Swiss albino mice twice daily with 10 mg/kg of glibenclamide (two groups of five animals) or 500 mg/kg glibenclamide (two groups of five animals). A control group of five animals received water only. The fasting plasma glucose was measured on day 0 (control group), and on days 3 and 5 (treated groups). In the Aloes groups, FPG levels were decreased after 5 days of treatment. The mice did not experience any adverse effects (Ghannam et al. 1986).

Hikino et al. (1986) reported that methanol-water extracts and water extracts of *Aloe arborescens* yielded two polysaccharide fractions, named arborans A and B. The arborans were administered intraperitoneally to normal mice (strain and number not given). It was found that both arborans decreased plasma glucose levels in a dose-dependent manner. In mice with alloxan-induced hyperglycemia, both arborans decreased plasma glucose levels.

Ajabnoor (1990) examined the possible hypoglycemic effect of aloe in hyperglycemic induced mice. Induction was via a single tail injection of alloxan monohydrate, 70 mg/kg. Male MFI mice were placed into four groups: a control group (0.9% saline 10 ml/kg, intraperitoneal injection); a reference group dosed with glibenclamide (10 mg/kg in 0.9% saline, oral administration); a third group dosed with the bitter principal from aloe (5 mg/kg in 0.9% saline, intraperitoneal injection); and a fourth group orally dosed with 500 mg/kg of aloe in 0.9% saline. All doses were a single administration. The 500 mg/kg dose of Aloe did not reduce the FPG, whereas the bitter principal reduced the FPG up to 24 h after administration. No mortality occurred during the study.

This author also conducted a 5-day study of the same design; however, the dosing was twice daily for 5 days. The twice-a-day dosing of aloe (500 mg/kg) decreased FPG starting on day 3, with the greatest decrease occurring by day 5. As in the single-administration study, the bitter principal significantly reduced FPG. No mortalities occurred over the 5-day study (Ajabnoor 1990).

Koo (1994) induced diabetes mellitus in male Sprague-Dawley rats by a subcutaneous injection of alloxan monohydrate (120 mg/kg) to study how a commercial health product containing *Aloe vera* gel would affect plasma glucose levels. One group of eight rats was treated with the *Aloe vera* gel preparation and another group of eight was treated with distilled water (both dosed once at 2 ml/kg). Three groups of 10 rats each were treated twice daily for 10 days. One group was orally administered the *Aloe vera* gel preparation, the second group was orally administered distilled water, and the third group received no treatment. None of the *Aloe vera* treated groups saw a decrease in their plasma glucose levels, although the levels were significantly increased compared to controls. The author concluded that an *Aloe vera* preparation is very likely to be ineffective in the treatment of diabetes mellitus.

Gastrointestinal Effects

Green (1941) tested aloin, isolated from *Aloe barbadensis* and *Aloe ferox*, against United States Pharmacopeia (USP) aloin for its effect on the large intestine of guinea pigs (strain and number not given). A 5-cm loop of large intestine was isolated from anaesthetized guinea pigs and injected with either 0.5 cc of USP aloin or aloin isolated from *Aloe barbadensis*. The concentrations of each injection were 1.0 to 1.5 mg/cc. Aloin extracted from *Aloe barbadensis* caused a higher degree of inflammation when compared to the USP aloin. Aloin extracted from *Aloe ferox* had the same degree of inflammation as the USP aloin.

Barbaloin, an anthrone C-glycoside found in aloe, sometimes referred to as vegetable laxative, is used as a treatment for constipation (de Witte 1993). Barbaloin decomposes to aloe-emodin-9-anthrone and aloe-emodin in the large intestine of rat, causing a significant increase in the water content. Ishii et al. (1990) found that aloe-emodin-9-anthrone inhibited rat colonic Na^+,K^+-adenosine triphosphatase (ATPase) in vitro and increased the paracellular permeability across the rat colonic mucosa. Thus the increased water content in the large intestine is due to multiple mechanisms of action of aloe-emodin-7-anthrone such as the inhibition of absorption and stimulation of secretion without stimulation of peristalsis.

WOUND HEALING

Aloe vera extract (preparation not given) was used to treat corneal ulcers in rabbits. The extract was applied as eye drops to the left eye of the rabbits while the right received saline. The *Aloe vera*–treated eyes had less signs of irritation, less cellular reaction, and more rapid healing (Hegazy et al. 1978).

Parmar et al. (1986) found both the exudate and the gel of *Aloe vera* did not provide a protective effect for gastric and duodenal ulcers. Koo (1994) confirmed the results of Parmar with oral administration of a health product containing *Aloe vera* gel to rats that did not protect against ulcer formation and did not promote healing of previously induced ulcers.

Contrary to these results, Saito et al. (1989) and Teradaira et al. (1993) found that extracts from *Aloe arborescens* do have suppressive and healing effects on gastric-induced ulcers in rats. Aloctin A (from *Aloe arborescens*) administered intravenously inhibited gastric juice, acid, and pepsin output in pylorus-ligated rats (Saito et al. 1989).

Davis et al (1994a) showed that mannose-6-phosphate, a major sugar in *Aloe* gel, was effective as an anti-inflammatory agent at 300 mg/kg in adult male ICR mice. Wound healing was also improved at this dose. Glucose-6-phosphate, another major sugar in *Aloe* gel, was not found to be effective.

Using HSD-ICR male mice Davis et al. (1994b) showed that mice receiving 1 mg/kg hydrocortisone had a 46.7% reduction in wound healing, but those treated with 100 or 300 mg/kg of aloe along with hydrocortisone showed 66.7% and 100% increase in wound healing, respectively. Sterols in aloe, especially lupeol, campesterol, and β-sitosterol, were found to be anti-inflammatory. The authors stated that aloe inhibited hydrocortisone's anti–wound-healing activity as well as increased wound tensile strength above the aqueous control. The authors proposed that the wound-healing properties of aloe could be attributed to stimulation of macrophages and fibroblasts.

Tizard et al. (1994) reported that 30 μg of freeze-dried acemannan (reconstituted in saline) injected at the site of wounds in young (3-month-old) F344 $\times$ BNF1 hybrid male rats decreased wound healing time of approximately 4 days when compared to untreated controls. When 2-year-old rats were treated in the similar manner, wound-healing time was decreased from 21 to 14 days. Acceleration of wound healing was also observed when Acemannan was administered by either intraperitoneal or intracardiac routes. The authors believe the effects of Acemannan are via activation of macrophages resulting in release of fibrogenic cytokines. The other possibility proposed by the authors was that the growth factors may bind directly to Acemannan, thereby increasing the stability and stimulating effects on granulation tissue formation.

Udupa et al. (1994) showed that in Wistar albino male and female rats, fresh juice (0.2 ml/100 g, intraperitoneal [IP]) of *Aloe vera* significantly increased breaking strength in skin and granuloma tissues, enhanced wound contraction and decreased epithelialization period. They also noted an increase in lysyl oxidase activity, mucopolysaccharide content in the wound area, and anti-inflammatory activity without much effect on chronic inflammation. The authors concluded that fresh juice of aloe could increase tensile strength by increasing cross-linking in collagen and interactions with the ground substance.

Roberts and Travis (1995) exposed the right leg of male C3H mice to single doses of gamma radiation ranging from 30 to 47.5 Gy. Acemannan-containing gel was applied beginning on day -7, 0, or $+7$ relative to the day of irradiation (day 0) and continuing for 1, 2, 3, 4, or 5 weeks. Unlike aloe-based ointments, wound-dressing gel maintains the healing properties associated with the fresh pulp of the plant but in a stable form with standardized potency. The right inner thigh was scored on a scale of 0 to 3.5 for severity of radiation reaction from the 7th to 35th days after irradiation. The left leg served as a control. The authors found that the average peak skin reaction of the wound-dressing gel–treated mice was lower than that of the untreated mice at all radiation doses. A reduction in skin reaction was greatest in the groups that received wound dressing for at least 2 weeks beginning immediately after irradiation. There was no significant effect if the gel was applied only before irradiation or beginning 1 week after irradiation. The authors also concluded that the Acemannan-containing wound-dressing gel, but not personal lubricating jelly or healing ointment, reduced acute radiation-induced skin reactions in C3H mice if applied daily for at least 2 weeks beginning immediately after irradiation.

Chithra et al. (1998) anesthetized male Wistar rats and cut a 2 $\times$ 2-cm wound extending into the subcutaneous tissue. *Aloe vera* gel was prepared from mature plants with the rind removed. Then the colorless parenchyma was ground in the blender and centrifuged at 10,000 *g* to remove fibers. This was lyophilized at room temperature. There were six rats per treatment: group I, untreated; group II, treated topically (30 mg/ml of *Aloe vera* in water); and group III, treated orally (gavage) with 30 mg/ml of *Aloe vera* in water. *Aloe vera* increased total collagen of the granulation tissue by 93% in topical treatments and 67% in the oral treatment. The degree of cross-linking increased as seen by an increase in aldehyde content (42% topical and 30% oral) and decrease in acid solubility for both treatment groups (5% topical and 4.8% oral). Type I/III collagen ratios of treatment groups were lower than the untreated controls, indicating enhanced levels of type III.

According to Reynolds and Dweck (1999), healing in rats and mice, wounded by punch biopsy, occurred more quickly when the animals were treated with decolorized *Aloe vera* gel (without anthraquinones). Daily injection of the *Aloe vera* gel reduced wound diameter and increased skin circulation. Topical application of Aloe gel healed cuts more rapidly than untreated controls or application of antimicrobials. Acemannan healed wounds of elderly and obese rats.

In rats and mice, *Aloe vera* has been used to decrease the inflammatory effects induced by kaolin, carrageenan, albumin, gelatin, mustard, and croton oil. Application of the *Aloe vera* gel was either topical or injected. Topically applied *Aloe vera* gel decreased croton oil–induced inflammation 67% in mice ears.

However, in pigs, *Aloe vera* had no effect on wound healing and no healing properties were seen in rabbits with corneal punch wounds (Reynolds and Dweck 1999).

Burn Healing

Robson et al. (1982) reported that a cream base containing 70% of *Aloe vera* extract was very effective in preserving the dermal microcirculation after thermal injury. It also inhibited some of the products of arachidonic acid metabolism such as thromboxane B_2 and limited the production of prostaglandin (PG) $F_{2\alpha}$, thus preventing progressive dermal ischemia. The authors concluded that *Aloe vera* extract was very beneficial in a burn wound.

McCauley et al. (1990) stated that *Aloe* vera can be used in the treatment of frostbite. The rationale was that both frostbite blisters and burns contain thromboxane and if *Aloe vera* reverses progressive ischemia in burns, it should also be effective for frostbite.

Aloe vera gel was tested in a variety of burn models in guinea pigs and rabbits (Reynolds and Dweck 1999). Burns induced by either heat or electrical means healed more rapidly with *Aloe vera* gel than with other treatment. *Aloe vera* gel was shown to penetrate tissue, relieve pain, reduce inflammation, and increase blood supply to the burn area. *Aloe vera* gel application to hot plate induced burns on guinea pig skin resulted in faster healing. In mice with gamma-radiation burns, *Aloe vera* gel preparations improved the recovery time of first- and second-degree burns.

Protection Against Aspirin-Induced Damage

Maze et al. (1997) reported that *Aloe vera* extracts provided protection against acute aspirin-induced mucosal injury in male Sprague-Dawley rats. Rats were fasted for 24-h and five per group were exposed: group 1 received 1 ml of saline by gavage followed 30 min later by gavage of 1 ml of vehicle; group 2 received 1 ml of *Aloe vera* extract (at a dose of 100 mg/kg body weight) by gavage followed 30 min later by gavage of 1 ml of vehicle; group 3 received 1 ml of saline by gavage followed 30 min later by gavage of 1 ml of aspirin (100 mg/kg); group 4 received 1 ml of *Aloe vera* extract (at a dose of 100 mg/kg body weight) by gavage followed 30 min later by gavage of 1 ml of aspirin (100 mg/kg); and group 5 received heat inactivated (boiled 30 min) 1 ml of *Aloe vera* extract (at a dose of 100 mg/kg body weight) by gavage followed 30 min later by gavage of 1 ml of aspirin (100 mg/kg). Pretreatment with *Aloe vera* extract reduced aspirin-induced acute gastric mucosal injury (recorded as number and severity of gross mucosal lesion in stomach and mucosal PGE_2 synthesis) by 70% ($p < .005$ versus saline pretreatment). Heat-inactivated aloe did not significantly reduce gastric lesions. Prostaglandin levels were not altered with pretreatment with native or heat inactivated *Aloe vera*.

Liver Effects

Arosio et al. (2000) divided 24 male Sprague-Dawley rats into four equal groups to investigate whether aloe-emodin has a protective effect on the liver. Group 1 was not treated. Group 2 was dosed with a single intraperitoneal injection of carbon tetrachloride (CCl_4) to induce liver damage. Group 3 received the CCl_4 injection, and an intraperitoneal injection of aloe-emodin (1 ml/kg) 2 hours before the CCl_4 injection and 2 hours after. Group 4 received only two aloe-emodin injections of 50 mg/kg each. All rats were killed within 24 h of dosing and their livers were examined. CCl_4 induced liver damage within 24 h in group 2. It also induced liver damage in group 3; however, the lesions were less marked. The control and group 4 livers did not have any lesions.

Norikura et al. (2002) reported that various solvent-extracted aloe powders (mixtures of *Aloe ferox* Miller, *Aloe africana* Miller, and *Aloe spicata* Baker) had a protective effect against 1,4-naphthoquinone and carbon tetrachloride hepatotoxicity in primary cultured rat hepatocytes (Sprague-Dawley). Cells incubated with 100 μg of 1,4-naphthoquinone (NQ) and 0, 25, 50, and 100 μg/ml of aloe had significant improvement of viability in a dose-dependent manner. Ethanol and ethyl acetate extracted aloe was the most protective ($p < .05$), whereas chloroform and butanol extracted aloe had no effect. Lactose dehydrogenase (LDH) activity decreased significantly. Glutathione (GSH) and thiol protein levels increased significantly with the treatment of ethanol and ethyl acetate aloe extracts on NQ-induced cells. Carbon tetrachloride (3 μl/2 ml)–induced cells had a significant increase in viability with the addition of 100 μg/ml of ethyl acetate extracted aloe ($p < .01$).

Woo et al. (2002) reported that aloe-emodin significantly inhibited the proliferation of activated primary hepatic stellate cells collected from Sprague-Dawley rats. The aloe-emodin exposures were 0, 1, 2.5, 5.0, and 10 μg/ml. Smooth muscle α-actin gene expression was inhibited and the production of collagen type I protein was decreased in a dose-dependent manner. Smooth muscle α-actin gene expression was inhibited 80 $\pm$ 10.7% (p0/01), and 99% ($p < .001$) of control levels by 5.0 and 10 μg/ml of aloe-emodin, respectively. Type I collagen was decreased to 59 $\pm$ 7% ($p < .05$), 37 $\pm$ 14% ($p < .001$), and 13 $\pm$ 12% ($p < .001$) of control levels by 2.5, 5.0, and 10 μg/ml of aloe-emodin respectively.

Hormonal Effects

Capasso et al. (1983) tested aloin for prostaglandin activity in the rat colon. Rats were treated with aloin (100 mg/kg) 4 h before sacrifice. Following death, the rat colons were excised, suspended in a medium of Tyrode solution for 30 min, and then assayed for prostaglandin activity. Colons from rats treated with aloin produced significantly more prostaglandin than the control group. Additionally, rats treated with indomethacin (4 mg/kg subcutaneous) 12, 24, and 48 h prior did not have an increase in prostaglandin production.

Kar et al. (2002) reported that *Aloe vera* gel was found to inhibit serum levels of thyroid hormones thyroxine (T_4) and triiodothyronine (T_3), thus suggesting their potential use as

TABLE 10
Summary of acute toxicity studies of injectable acemannan (Fogelman et al. 1992a)

Species	No. of each sex	Route	Dose (mg/kg)	Result	LD_{50}
Mouse	5	IV	20, 40, 80	No effects	>80 mg/kg
	5	IP	100, 200	No effects	>200 mg/kg
Rat	5	IV	7.5, 15	No effects	>15 mg/kg
	5	IP	25, 50	No effects	>50 mg/kg
Dog	2	IV	10	Emesis, loose stool in 2/4, 2–4 h post dose	>10 mg/kg
	2	IP	50	Emesis, loose stool, decreased activity, 2–4 h post dose	>50 mg/kg

antithyroidal agents. The *Aloe vera* gel was prepared by removing the outer rind and the colorless parenchyma was ground in a blender and centrifuged to remove fibers. Swiss albino male mice were dosed with 125 mg/kg of *Aloe vera* by gastric intubation. Levels of hepatic lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) were not affected by administration of 125 mg/kg of *Aloe vera* as compared to vehicle controls.

Hypotensive Effect

Saleem et al. (2001) reported that aloe-emodin and aloin A extracted from *Aloe barbadensis* had a hypotensive effect on Sprague-Dawley rats. The mean arterial blood pressure was reduced by 26%, 52%, and 79% at corresponding doses of 0.5, 1, and 3 mg/kg.

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Shah et al. (1989) administered *Aloe vera* extracted in ethanol orally to Swiss albino male mice at doses of 500 mg/kg, 1 g/kg, and 3 g/kg. No signs of toxicity were observed, except in the higher dose groups, in which there was a decrease in central nervous system (CNS) activity noted (CNS activity measurement not reported).

Lagarto Parra et al. (2001) administered an aloe extract to Swiss albino mice (number not given) for estimation of LD_{50}. *Aloe vera* leaves were dried, chopped, and extracted in methanol. The extracts (concentrations were not given) were given orally. The estimated LD_{50} for *Aloe vera* 24 h after dosing was 120.65 mg/kg.

MDS Pharm Services (2000) reported a study in which Aloe Barbadensis Leaf Water (2.01 ml volume of 100% Aloe) was administered by gavage to 10 Sprague-Dawley rats (5 females, 5 males). There were no clinical signs of toxicity, mortality, weight changes, and no gross findings at necropsy (at day 14).

Acute Parenteral Toxicity

Dhar et al. (1968) conducted a study using the entire *Aloe barbadensis* plant, ground up into a powder, extracted with 50% ethanol, and dried. The dried extract was suspended in either 0.1% agar solution or 1.0% gum acacia in distilled water. This extract was administered intraperitoneally to adult albino mice (either sex, two to three animals) at an initial dose of 400 to 500 mg/kg. The dose was increased or decreased by a factor of two. The maximum tolerated dose (MTD) was 100 mg/kg body weight and the LD_{50} was 250 mg/kg.

Fogelman et al. (1992a) administered acemannan (in saline) once intravenously by tail vein to Sprague-Dawley rats and CD-1 mice or by cephalic vein to purebred Beagle dogs or by intraperitoneal injection to rats, mice, and dogs at volumes considered to be maximal without undue discomfort and that did not interfere with the interpretation of results. Each animal was observed during the 1st hour following dosing, at 4 h, and daily thereafter for 14 days. Body weights were recorded. All animals surviving to day 14 were euthanized and necropsied. Organ weights were recorded for liver, kidney, pancreas, thymus, thyroid, and spleen. No significant adverse effects, deaths or gross pathology occurred in any of the animals. Study results are summarized in Table 10.

Acute Dermal Toxicity

Aloe Ferox Leaf Extract was applied to shaved skin of male white New Zealand rabbits. The shaved skin was scarified (excision with a scalpel cutting the epidermis only) or not damaged. The two skin sites were exposed to 0.5 ml of the products and occluded with hydrophilic patches. One of the six rabbits had a reaction of a very slight erythema on the scarified tissue that cleared after 72 h and Aloe Ferox Leaf Extract was considered a nonirritant (0.04:8) (Institut Francais de Recherches et Essais Biologoques 1981).

Short-Term Oral Toxicity

Ajabnoor (1990) reported that male albino mice (MFI strain) were orally administered the bitter principal of Aloes in 0.9% saline (up to 20 mg/kg) for 2 weeks. Further experimental details were not provided. No mortality was observed.

Fogelman et al. (1992b) mixed acemannan in the basal diet of Sprague-Dawley rats at dose levels of 0, 6000, 12,500, 25,000,

or 50,000 ppm for 2 weeks. Animals were observed twice daily. Five randomly selected rats/sex/group were evaluated for hematologic and serum chemistry parameters at termination (day 14). All survivors were necropsied and brain, heart, liver, kidney, adrenals, and testes weights were recorded. No mortalities occurred. Body weights, body weight gains, and food consumption were comparable in all groups. Hematology and serum chemistry values were comparable between treated and control animals. At necropsy, three males in the 12,500 ppm dose group had red/brown foci on the stomach; all other males were normal. A female in the 50,000 ppm group had a reddened stomach. These observations were sporadic and not dose-related. Absolute and relative organ weights were not significantly different from controls. The 14-day NOEL (no observed effect level) for acemannan in the diet, in rats, was 50,000 ppm, equivalent to 4068 mg acemannan/kg/day in male rats and 4570 mg acemannan/kg/day in female rats.

Short-Term Parenteral Toxicity

Male albino mice (MFI strain) were administered the bitter principal of Aloes in 0.9% saline (up to 20 mg/kg) intraperitoneally for 2 weeks. No mortalities were observed (Ajabnoor 1990).

Fogelman et al. (1992a) reported that no significant toxicities were seen in rats, mice, and dogs after eight doses of acemannan (in saline) administered intravenously or intraperitoneally at four-day intervals over 30 days. The results are summarized in Table 10.

Dose levels were 4 to 50 mg/kg for Sprague-Dawley rats, 20 to 200 mg/kg for CD-1 mice, and 0.3 to 50 mg/kg for Beagle dogs. Rats and mice were distributed into groups of 10 per sex and Beagle dogs were distributed into groups of 2 per sex. All animals were observed daily, and 1 and 4 hours after dosing. Body weights for dogs were recorded prior to each injection and weekly for the rats and mice. Food consumption was measured weekly for rats and mice. In addition, all animals had ophthalmologic examinations prior to study initiation and at termination. All animals had hematologic and serum chemistry evaluations. Tissues obtained at necropsy were examined. In addition, select organ weights were recorded.

Of mice intravenously administered acemannan, 3/20 at the 40 mg/kg/day and 6/20 at the 80 mg/kg/day dose level died. Three of these deaths were judged to be unrelated to the treatment. Clinical signs seen in the mice were limited to decreased activity, abnormal gait and stance, flaccid body tone, piloerection, and tremors. These signs appeared to be transient as recovery was apparent within 24 h. Mean body weights of the 80 mg/kg/day acemannan group were significantly lower than controls on days 8, 15, and 22. Hematology and serum chemistry values were comparable to controls. Treatment related histological changes were found in the lungs at all dose levels and were characterized as minimal to slight multifocal accumulations of macrophages.

Acemannan administered intravenously to rats did not affect body weight and food consumption or produce any clinical signs of toxicity. Three rats died while on study but the cause of death was not apparent. Histologically, the test rats had the same observations in the lungs as the mice.

Body weights of dogs treated intravenously with acemannan were not affected. Clinical signs were limited to emesis seen sporadically among the groups. Hematologic and serum chemistry values were within normal ranges for Beagles (compared to historical data). Histological findings in the dogs treated intravenously were similar to those seen in the rats and mice.

In both rats and mice (intraperitoneal administration), body weights and food consumption were similar to the controls. Clinical signs of toxicity were not seen in the rats; however, the mice had abnormal gait and stance, poor grooming, piloerection, and ptosis. Five mice died from treatment related causes; no rats died while on study. In the rats and mice, hematology and serum chemistry values were generally similar to the controls. However, male mice at the 100 mg/kg/day dose and females at the 200 mg/kg/day dose levels had a significant increase in total leukocytes. Significant differences in absolute or relative organ weights were seen in the mid- and high-dose male mice, high-dose female mice, and the mid- and high-dose female rats. Histological changes for both the mice and rats were limited to microgranulomas seen on the surfaces of the peritoneal organs.

As seen in the intravenous study, dogs treated with acemannan intraperitoneally did not have any changes in their body weights. The intraperitoneally treated dogs experienced emesis, decreased activity, and abdominal discomfort. Hematology and serum chemistry values were not significantly different from controls; however, there were increases in the monocyte levels. No significant differences occurred in organ weights of the treated dogs. Histological responses were similar to those seen in the rats and mice.

Maximum dose levels with no observed adverse effects were 20 mg/kg intravenous or intraperitoneal in mice, and 4 mg/kg intravenous and 50 mg/kg intraperitoneal in rats. The no adverse effect level for dogs was observed with 1 mg/kg intravenous and the lowest observed adverse effect level for intraperitoneal administration was 5 mg/kg (Fogelman et al. 1992a).

Subchronic Oral Toxicity

Shah et al. (1989) conducted a study in which 20 Swiss albino male mice were orally administered *Aloe vera* (extracted in ethanol) in their drinking water at a dose of 100 mg/kg for 3 months. Control animals were dosed with distilled water. All animals were observed for signs of toxicity and mortality, and body weights were determined during the study. Blood and organs were analyzed from five animals in the control and *Aloe vera* treatment groups at study termination. Body weights and organ weights in treated animals were not different from controls. No abnormalities of the viscera were observed in treated or control animals. Six of the twenty *Aloe vera*–treated mice

died while on study; this was significant when compared to the controls. Alopecia of the genital region and degeneration and putrification of the sex organs occurred in 20% of the animals. Ten percent of the animals had inflammation of the hind limb. Red blood cell counts were considerably decreased in the *Aloe vera*–treated animals.

Fogelman et al. (1992b) mixed acemannan in a predetermined quantity of feed to deliver doses of 0, 100, 400, or 1500 mg/kg/day for each purebred Beagle dog over a 90-day dosing period. The dogs were observed twice daily for clinical signs of toxicity. Detailed examinations were done prior to initiation of dosing and at weeks 4, 5, 8, 9, 10, 11, 12, and 13. Body weights were recorded weekly. Ophthalmic examinations were conducted prior to study initiation and at termination of the study. Serum chemistry, hematologic, and urinalysis data were recorded at day 45 and at termination. The animals were euthanized at day 90 and subjected to a complete necropsy.

There were no significant signs of systemic toxicity. Body weights and food consumption were comparable between treated and nontreated dogs. Serum chemistry, hematology, and urinalysis data from the treated dogs were all within the normal limits at all times evaluated. No significant gross or microscopic lesions were attributed to the ingestion of acemannan. The NOEL for acemannan, orally administered to Beagle dogs, was at least 1500 mg/kg/day (estimated), which is equivalent to 1,170 mg acemannan/kg/day.

In a six month study by these authors, acemannan was mixed in the basal diet of Sprague-Dawley (20 rats/sex/group) rats to provide acemannan doses of 0, 200, 650, or 2000 mg/kg/day. Individual body weights were determined initially, weekly for 15 weeks, biweekly thereafter, and at study termination. Food consumption was measured during the week prior to initiation and concurrently with body weights throughout the study. Twice daily observations were made for mortality and clinical signs of toxicity. Concurrent with the body weight data collection, detailed physical examinations were performed. Ophthalmic examinations were performed initially and at study termination. At 1, 3, and 6 months, 10 rats/sex/group underwent hematology, serum chemistry, and urinalysis determinations. Complete gross necropsy examinations were conducted on all animals that died during the study, 10 rats/sex/group at day 90, and all survivors at 6 months. Organ weight data were obtained and select tissues from the control and high-dose groups were examined.

Body weights for males and females receiving acemannan were comparable to controls throughout the study. Male acemannan consumption ranged from 193 to1986.3 mg/kg/day and female acemannan consumption ranged from 200.9 to 1994.8 mg/kg/day. Adverse clinical signs were randomly distributed among the dosage groups and sexes, and were not considered to be due to acemannan. Ophthalmological examinations revealed no significant changes among the rats. All serum chemistry, urinalysis, and hematological values were normal. Organ weights and gross and microscopic pathology for the treated rats were normal and similar to the corresponding controls. No gross lesions attributable to the ingestion of acemannan were noted in any of the rats.

Six female rats died during the study; four deaths were attributed to the bleeding procedure. Of the two unexplained deaths, one female at the 2000 mg/kg/day dose died on day 30 and the gross necropsy revealed enlarged and mottled kidneys. The other unexplained death occurred on day 139 in a female receiving the 650 mg/kg/day dose. The cause of death was confirmed histologically as chronic pyelonephritis. The 6-month NOEL in rats was 2000 mg/kg/day, which was equivalent to 1549 mg acemannan/kg/day for males and 1555 mg acemannan/kg/day for females (Fogelman et al. 1992b).

Herlihy et al. (1998a) administered *Aloe barbadensis* to four groups of SPF Fischer 344 male rats (20 rats per group) for 1.5 or 5.5 months. *Aloe barbadensis* was processed into four formulations. Formulation A1 was a 1% preparation of Aloe filets that had been homogenized, lyophilized, and frozen. Formulation A10 was the same preparation, at a 10% concentration. B1 and B10 formulations were prepared the same way as A1 and A10; however, the homogenate was charcoal filtered prior to lyophilization (removal of barbaloin) to more closely resemble commercial products. The four groups of rats received one of the Aloe preparations in their diet; a fifth, the control group, was fed normal rat chow. Data were collected for food consumption, water consumption, body weights, clinical signs, blood chemistry, histopathological analysis, and gastrointestinal transit time.

Due to diarrhea and restricted growth rates seen in the rats fed A10, this group was eliminated from the study. Formulations A1, B1, and B10 did not have any effect on body weights, food consumption, organ weights, or gastrointestinal transit time when compared to the controls. B10 increased water consumption; however, analysis of the rat chow/Aloe mixture revealed increased salt levels that arose from the concentration of the salts during lyophilization. In the groups fed the Aloe preparations, pathology and blood chemistry were not significantly different from the controls, except for decreased serum cholesterol, HDL, aspartate transferase, and alanine transferase (Herlihy et al. 1998a).

Ocular Toxicity

Aloe Ferox Leaf Extract was instilled in the conjunctival sac of the eye of male New Zealand white rabbits. Six healthy male rabbits received 0.1 ml (no concentration specified) of Aloe Ferox Leaf Extract in the conjunctival sac of the eye and were observed 1 h, 24 h, 2 days, 3 days, and 7 days after administration. No significant changes were seen except for 5/110 combined Draize score after 1 h (all six animals exhibited minimal chemosis and 5/6 had slight corneal erythema), a 2/110 combined Draize score after 1 day, and 0/110 after day 2 (Institut Francais de Recherches et Essais Biologoques 1981).

The Institut D'Expertise Clinique (2000) administered Aloe Barbadensis Leaf Water (300 μl; no concentration specified) to the vascular chorionic-allantoic membrane of White Leghorn

PA12 eggs in an alternative ocular irritation assay. After contact for 20 s, the membrane was washed with 5 ml of physiological serum. There were four eggs per treatment; a negative control of 0.9% physiological serum; and positive control of sodium dodecyl sulfate (SDS) 0.5% w/v. The mean irritation index for SDS was 12.0 (irritant) and the Aloe irritation index was 1.3 (slight irritant).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Shah et al. (1989) reported that Swiss albino male mice were orally administered *Aloe vera* (extracted in methanol) in their drinking water for three months at a dose of 100 mg/kg. The extracted *Aloe vera* caused significant sperm damage (megacephali and flat head > swollen achrosome > rotated head) when compared to the controls (dosed with distilled water).

Parry and Matambo (1992) studied the reproductive toxicity of three Aloe species (*Aloe chabaudii*, *Aloe globuligemma*, *Aloe cryptopoda*) in Wistar rats and mice (strain not given). No information was available on the similarity of extracts from these species and those addressed in this report. The three Aloe plants were prepared the same way: pounded with a mortar and pestle, collection of the juices, filtered through muslin cloth, and freeze dried. The dried powder was reconstituted with distilled water. Ten rats received 500 mg/kg of *A. chabaudii* (eight were dosed intraperitoneally and two were dosed orally) on days 14, 15, and 16 of their pregnancy. Eight rats received 500 mg/kg of *A. cryptopoda* intraperitoneally and eight rats received 500 mg/kg intraperitoneally of *A. globuligemma*. Control rats were administered saline intraperitoneally. The mice were only administered *A. chabaudii* either orally (four) or intraperitoneally (five) (500 mg/kg) on days 14, 15, and 16 of their pregnancy. Control groups received normal saline either intraperitoneally or orally.

Even though a number of rats died due to the toxicity of the Aloe, no resorptions of the fetuses occurred. Additionally, no abortions were noted. Rats and mice that survived delivered normal sized, healthy litters (Parry and Matambo 1992).

Nath et al. (1992) extracted ground up leaves of *Aloe barbadensis* with water and orally administered (vehicle was 1% acacia) the material to five pregnant Charles Foster albino rats. Dose administration took place from gestation day 0 though gestation day nine. Five control rats were given the vehicle only. Cesarean delivery was performed on gestation day 20. A total of 51 fetuses was examined for macroscopic effects, 25 for visceral abnormalities, and 26 for skeletal abnormalities.

In the treatment group, per animal, the implantations were 13.0 ± 1.0 (8.6 ± 1.1 in controls); resorptions were 2.8 ± 1.1 (none in controls); live births were 10.2 ± 1.3 (8.6 ± 1.1 in controls); fetal body weight was 2.60 ± 0.85 (4.83 ± 0.44 in controls); and fetal body length was 2.87 ± 0.42 (3.87 ± 0.22 in controls). Overall 21.5% abortifacient activity (resorptions/implantations) was calculated (0% in controls). No macroscopic, visceral, or skeletal deformities were reported in control fetuses. Macroscopic effects in treatment fetuses included kinking of tail (5.9%), clubbing of right hind limb (11.8%), and left wrist drop (19.6%). No visceral abnormalities were seen in treatment fetuses. Skeletal deformities in the treatment fetuses included nonossification of skull bones (15.4%), nonossified ribs (15.4%), fused tarsal (15.4%), and intercostal space in ribs (11.5%). The authors noted that traditional medicinal plants used as abortifacient agents can have an effect on the fetus if not 100% effective (Nath et al. 1992).

GENOTOXICITY

Both negative and positive results were found in bacterial and mammalian cell genotoxicity assays using *Aloe barbadensis*, *Aloe ferox* and various components of Aloe. The data are summarized in Table 11.

CARCINOGENICITY

Siegers et al. (1993) reported that male NMRI mice were administered different diets to test if aloin was carcinogenic. The subjects were randomized into five groups (20 per group), and each was fed a different diet for 20 weeks. Group 1 was injected subcutaneously with 20 mg/kg of dimethylhydrazine (DMH) to induce tumors weekly for 10 weeks plus 0.03% (equivalent to 100 mg/kg/day) sennosides in diet for 20 weeks. The second group was injected subcutaneously with 10 mg/kg of EDTA (solvent for carcinogen) plus 0.03% sennosides in diet for 20 weeks. Group 3 was injected subcutaneously with 20 mg/kg DMH weekly for 10 weeks plus normal diet for 20 weeks. Group 4 was injected subcutaneously with 20 mg/kg DMH weekly for 10 weeks plus 0.03% aloin in diet for 20 weeks. The final group was injected subcutaneously 10 mg/kg EDTA weekly for 10 weeks plus 0.03% aloin in diet for 20 weeks. The mice were sacrificed by decapitation at the end of the experimental period. Tumors were detected only in the distal segment of the colon and rectum.

The authors concluded that aloin did not significantly alter the incidence of colorectal tumors. Group 3 (normal diet plus DMH) produced 10/19 tumor-bearing animals, group 4 (aloin plus DMH) had 7/20 tumor-bearing animals, and group 5 (control for aloin) had 1/ 20 tumor-bearing animals. The mean tumor rate in tumor-bearing animals was 1.50 ± 0.22, 1.0, and 2.0 ± 0.58 for groups 1, 2, and 3, respectively (Siegers et al. 1993).

Strickland et al. (2000) reported a study in which semisynthetic and extracted aloe-emodin (from *Aloe barbadensis*), in 25% ethanol, were applied to specific pathogen free female C3H/HeNCr (MTC^-) mice. Mice were exposed to 15 kJ/m^2 UVB radiation three times weekly. Treatment was with either aloe-emodin or 25% ethanol (1 ml per mouse) and took place before UVB exposure. Control mice were not irradiated but were treated with either aloe-emodins or ethanol. Two additional groups were treated with either a total of 90 kJ/m^2 UV radiation for 2 weeks then aloe-emodin three times weekly for 31 weeks or a total of 60 μg aloe-emodin and then UV radiation for 31 weeks. The mice administered either aloe-emodin

TABLE 11
Genotoxicity of aloe compounds

Material tested	Assay type	Treatment	Results	Reference
		Bacterial Cell Assays		
Aloe-emodin	*Salmonella* TA1537	With and without activation, 100 μg/plate	61 *His* revertants per plate, 0.22 per nmole of aloe emodin; activation had no effect	Brown et al. 1977
Aloe-emodin		With and without activation, 50 μg/plate	330 *His* revertants per plate, 0.42 per nmole of emodin; significant activation	
Aloe-emodin	*Salmonella* TA1535, TA100, TA1537, TA153, TA1538, TA98	With and without S9 activation	Negative in all strains except for TA1537 without S9	Brown and Dietrich 1979
Aloin	*Salmonella*/ microsome TA1535, TA100, TA1537 TA1538, TA98	With and without S9, 50–250 μg/plate	Negative all strains	Brown and Dietrich 1979
Aloe ferox water extracted	*Bacillus subtilis* rec-assay	With and without metabolic activation concentrations of 5, 10, 20, 50, 100 mg/ml	Positive	Morimoto et al. 1982
	Salmonella TA98, TA100		Negative	
Aloe ferox ethanol extracted	*Bacillus subtilis* rec-assay	"	Negative	
	Salmonella TA98, TA100		Negative	
Aloe-emodin	*Salmonella* TA1537, TA1538, TA98	With and without activation 1–1000 μg/ml	Mutagenic in all strains with and without activation	Westendorf et al. 1990
Aloe-emodin	*Salmonella* TA 1537, TA1538, TA98	10–5000 μg/plate with/without S9	TA 1537 and TA 98 negative with S9, positive without S9; TA1538 positive with/without S9	Heidemaan et al. 1993
Aloe-emodin	Micronucleus test	NMRI mice, 1500 mg/kg	Negative	Heidemaan et al. 1993
Aloe-emodin	Chromosome Aberration test	Wistar Rats, 2000 mg/kg	Negative	Heidemaan et al. 1993
Crushed leaves of *Aloe barbadensis*	*Salmonella* TA100, TA98	TA98 with and without metabolic activation, 6 mg/ml	All strains negative	Badria 1994

TABLE 11
Genotoxicity of aloe compounds *(Continued)*

Material tested	Assay type	Treatment	Results	Reference
Aloe-emodin	*Salmonella* TA1535, TA1537, TA1538, TA98	10–5000 μg/plate with or without S9	TA1535 negative with/without S9; TA1537 and TA 98 negative with S9, positive without S9; TA1538 positive with/without S9	Heidemaan et al. 1996
		Mammalian Cell Assays		
Aloe-emodin	C3H/M2 fibroblasts	1–30 μg/ml	Positive reaction in transformation of cells	Westendorf et al. 1990
Aloe-emodin	V79-HGPRT assay	5–30 μg/ml	Weakly mutagenic	Westendorf et al. 1990
Aloe-emodin	Chromosome Aberration test	CHO cells with and without S9, at 18.75–75.00 μg/ml	With S9, positive at 37–75 μg/ml Without S9, positive at 18.75–75 μg/ml	Heidemaan et al. 1993
Aloe-emodin	Mouse TFT-resistance L5178Y $tk^{+/-}$ mutation assay	37–111 μM	tk mutations were induced in mouse lymphoma cells L5178Y	Mueller et al. 1996
Aloe-emodin	V79-HGPRT	1.6×10^6 cells exposed with and without S9, 5–350 μg/ml	Negative with/without S9	Heidemaan et al. 1996
		Animal Assays		
Aloe-emodin	Mouse Spot test	NMRI females crossed with DBA males given 200 and 2000 mg/kg on gestation day 9	Negative	Heidemaan et al. 1996
Aloe-emodin	Micronucleus test	NMRI mice, 1500 mg/kg	Negative	Heidemaan et al. 1996
Aloe-emodin	Chromosome Aberration test	Wistar rats, 2000 mg/kg	Negative	Heidemaan et al. 1996
		In Vitro Assays		
Aloe-emodin	UDS test	In vitro and in vivo in Wistar rats, 100 and 1000 mg/kg	Negative	Heidemaan et al. 1993

alone or vehicle alone did not develop tumors by study termination. Sixteen out of 20 mice dosed with either 2 μg or 5 μg of either aloe-emodin and then UV radiation had tumors by study termination; however, the number was not significantly different from the ethanol-treated/UV-radiated mice. However, aloe-emodin significantly increased the incidence of melanoma tumors when compared to the unirradiated controls. Mice that were exposed to UV radiation first and then aloe-emodin did not develop tumors. Mice that were exposed to aloe-emodin and then the UV radiation did develop tumors, 30% of which were melanoma.

Anticarcinogenicity

Aloe barbadensis

Harris et al. (1991) administered acemannan to 32 dogs and 11 cats that had spontaneous malignant tumors that had failed conventional treatment. Each animal received 1 mg/kg of acemannan intraperitoneally and 2 mg of acemannan injected into

TABLE 12
Effect of acemannan on fibrosarcomas (King et al. 1995)

Species	Age	Sex	Results	Tumor-free interval[a]	Survival (days)[b]
Canine	3	M	Free of disease	408+	442+
Canine	7	F	Free of disease	559+	603+
Canine	7	M	Died post surgery	0	52
Canine	8	F	Free of disease	409+	440+
Canine	10	F	No viable tumor present at time of surgery	303	303
Canine	12	M	Died week 11, recurrent disease	77	144
Canine	12	F	Free of disease	475+	498+
Canine	13	F	Died; recurrent tumor outside radiation field	162	225
Feline	6	M	Euthanized; recurrent tumor	204	246
Feline	7	M	Free of disease	555+	594+
Feline	8.5	F	Free of disease	428+	467
Feline	12	M	Free of disease	450+	472+
Feline	13	F	Died post surgery, no viable tumor present at time of surgery	0	97

[a]Time from surgical intervention.
[b]Time from study entry.

the tumor, if possible. Treatment occurred every 3 weeks for a total of five treatments. Thirteen of the 43 animals had no significant clinical or histopathological response. Among the 13 animals, 5 were lost to follow-up, 7 died after receiving one injection, and 1 had no response after a full course of therapy. Twelve animals had obvious signs of clinical improvement. Improvement was based on tumor shrinkage, tumor necrosis, or an unexpected long survival period. No toxicity was associated with intraperitoneal administration of acemannan.

Peng et al. (1991) reported that acemannan, from *Aloe barbadensis*, initiated the production of monokines that supported antibody dependent cellular cytotoxicity and stimulated blastogenesis in thymocytes. Additionally, acemannan, in both enriched and highly purified forms, was administered intraperitoneally to female CFW mice (number not given) into which murine sarcoma cells had been previously subcutaneously implanted. The highly malignant sarcoma cells grew in 100% of the implanted control animals resulting in mortality within 20 to 46 days. In the animals treated with acemannan at implantation, approximately 40% survived. The tumors from the acemannan-treated animals had vascular congestion, edema, polymorphonuclear leukocyte infiltration, and central necrosing foci with hemorrhage and peripheral fibrosis.

Sheets et al. (1991) administered acemannan intraperitoneally to cats diagnosed with feline leukemia. Forty-four cats were dosed for 6 weeks at 2 mg/kg and observed for an additional 6 weeks after the end of dose administration. At the end of the 12-week period, 29/41 cats were still alive. Two of the original cats were lost in follow-up and 1 died of other causes; the other 15 died of feline leukemia. Two months after the completion of the study the owners of 22 cats were interviewed. Twenty-one of those cats were still alive, and according to the owners, happy and healthy pets.

King et al. (1995) treated eight dogs and five cats with new or recurring fibrosarcomas with acemannan. All animals received 1 mg/kg body weight of acemannan weekly for 6 weeks, followed by monthly injections for 1 year. Prior to surgery, 2 mg of acemannan was injected into the tumor weekly for up to 6 weeks. Between the 4th and 7th weeks following initiation of intraperitoneal injections, surgical incision was attempted on the tumors. All animals that survived surgery immediately underwent radiation therapy. Of the 13 animals in the study, 7 survived and remained tumor free. Mean survival time was 240 days. Of the six animals that died, two died from postsurgical complications and one died from other causes. Study results are summarized in Table 12.

Kim and Lee (1997) tested aloe gel extracts from *Aloe barbadensis* for their chemopreventive capabilities in vitro and in vivo. Rat hepatocytes treated with [^{3}H]benzo[a]pyrene in the absence and presence of the Aloe gel extracts (0 to 250 μg/ml) had a dose-related inhibition of [^{3}H]benzo[a]pyrene adduct formation (9.1% to 47.7%) in the Aloe gel extracts group. No cytotoxic effects on rat hepatocytes were observed. Aloe gel extracts (250 μg/ml) and [^{3}H]benzo[a]pyrene incubated with rat hepatocytes for 3 to 48 h had a slight inhibitory effect of Aloe gel extracts on DNA adduct formation at 3 h with maximum inhibition occurring at 6 h (36%). Male ICR mice (18 mice/group) were assigned to the following groups: group 1,

control; group 2, daily oral treatment with 50 mg/mouse/day of Aloe gel extracts in distilled water; group 3, single oral dose of benzo[a]pyrene (10 mg/mouse in corn oil); group 4, single oral dose of benzo[a]pyrene (10 mg/mouse in corn oil) then daily oral treatment with Aloe gel extracts (10 mg/mouse/day) for 16 days; and group 5, oral pretreatment with Aloe gel extracts (10 mg/mouse/day) for 16 days followed by a single oral dose of benzo[a]pyrene (10 mg/mouse in corn oil) and then continuous treatment with 50 mg/mouse/day of Aloe gel extract for 16 days.

DNA adduct formation was significantly decreased in groups 4 and 5 when compared to group 3. Glutathione *S*-transferase activity was slightly increased in the liver but cytochrome P450 content was not affected by Aloe gel extract. The authors concluded that the inhibitory effect of the Aloe gel extract on benzo[a]pyrene diol epoxide–DNA adduct formation might have a chemopreventive effect by inhibition of benzo[a]pyrene absorption (Kim and Lee 1997).

Corsi et al. (1998) conducted a study in which male Fisher rats (number not given) previously injected with Yoshida AH-130 ascite hepatoma cells (2×10^5 in a saline suspension) into the pleural space received *Aloe vera* (20 μg suspended in 0.5 ml sterile saline) injections daily for 2 weeks. Injections of *Aloe vera* were also administered into the pleural space. At specified times (days 7 and 14) the growth of the tumor cells in the pleural space was evaluated. The use of *Aloe vera* significantly decreased tumor cell growth with respect to nontreated control animals. The survival of rats was progressively prolonged when *Aloe vera* was used as a therapy in the tumor-bearing animals.

Shamaan et al. (1998) studied the effects of an aloe derivative in rats. Male Sprague-Dawley rats were divided into six groups of five to eight rats per group. Groups 1 to 3 received a basal diet: group 1 was the control group and received no additional treatment; group 2 received vitamin C in their drinking water; group 3 received 0.1 g *Aloe vera* gel extract/100 ml in their drinking water for the 9-month study. A single dose of diethylnitrosamine (DEN) 200 mg/kg body weight and a diet containing 0.02% (*w*/*w*) 2-acetylaminofluorene (AAF)-induced chemical hepatocarcinogenesis in groups 4 to 6. Group 5 received vitamin C and group 6 received *Aloe vera* gel extract as previously described. DEN/AAF treatment significantly increased the GGT (γ-glutamyltransferase) concentration in carcinogen-induced rats; however, the addition of *Aloe vera* gel extract significantly decreased the activity of GGT. Both GGT- and GSTP (placental glutathione *S*-transferase)-positive foci were detected in the carcinogen treated rats; however, the addition of *Aloe vera* gel extract to the drinking water of group 6 caused a significant decrease in number of foci on their livers. The livers of the rats in group 6 were devoid of neoplastic nodules whereas cellular damage was seen in group 4. *Aloe vera* gel extract supplementation was found to be able to reduce the severity of chemical hepatocarcinogenesis.

Kim et al. (1999) screened a polysaccharide of Aloe gel extract of *Aloe barbadensis* for its chemopreventive effects using biomarkers involved in chemical carcinogenesis. In the benzo[a]pyrene (B[a]P)-DNA binding assay, a concentration dependent inhibition of DNA adduct formation occurred with the Aloe polysaccharide (180 μg/ml). Oxidative DNA damage by 8-hydroxyoxyguanosine was significantly decreased by the Aloe polysaccharide (180 μg/ml). Additionally, the Aloe polysaccharide had a positive effect in a dose-dependent manner on inhibition of phorbol myristic acetate (PMA)-induced tyrosine kinase activity in HL-60 cells and PMA-induced ornithine decarboxylase activity in BALB/3T3 cells. The Aloe polysaccharide significantly inhibited superoxide anion formation.

Pecere et al. (2000) screened aloe-emodin in vitro and in vivo for its anticarcinogenicity activity against highly malignant tumors: neuroectodermal tumors, Ewing's sarcoma, and neuroblastoma. The in vitro study was conducted first culturing neuroblastoma cells, pPNET cells, Ewing's sarcoma cells, T-cell leukemia cells, vinblastine-resistant cells, colon adenocarcinoma cells, and doxorubicin-resistant cells. Cytotoxicity activity was determined by growing cells in the presence of aloe-emodin for 72 h, using many different concentrations of aloe-emodin. After 24 and 48 h, the cells were scraped, washed, fixed overnight in a buffer solution, and processed for viewing. The results showed that aloe-emodin decreased the survival of tumor cells and normal fibroblasts. There was a large difference, however, between the two. The reduction in the neuroectodermal tumor cell lines was much more significant than in the different tumor cell lines and normal fibroblasts.

The results of the in vitro study led to another antitumor study that used 6-week-old female severely compromised immunodeficient (SCID) mice. The mice were organized into three different groups. The first two groups both received IP injections in the dorsal region of 10×10^6 human neuroblastoma cells; however, the difference between the two groups was when they started treatment. Drug treatment started immediately in the first group that continued for 5 days for a total of 5 doses. Five animals received 0.4 ml/day of DMSO (saline solution—control), and the other mice were treated with 50 mg/kg/dose of aloe-emodin. The second group received the same doses, except treatment did not start until 15 days after they were injected with tumor cells. All of the mice were sacrificed in groups 1 and 2 when the mean tumor volume in the control was 1.5 cm^3. In group 3, the treatment was exactly the same as group 1; however, these mice received the same amount of IP injections of colorectal adenocarcinoma a cells instead of neuroblastoma cells. All of the mice were sacrificed at day 30. In all three groups, the tumors were measured with a micrometer caliper twice a week throughout the study.

The tumors in group 1 (immediate treatment of aloe-emodin) were very sensitive to the drug, showing a significant reduction of growth in the animal hosts. When aloe-emodin treatment was delayed (group 2) until a palpable tumor mass had developed (day 15), tumor growth was halted throughout the period of drug administration. The group that was injected with colorectal adenocarcinoma cells was refractory to the treatment. No appreciable signs of acute or chronic toxicity were observed

in any of the treated animals; weight, neurological and intestinal functions, and hematological parameters were normal (Pecere et al. 2000).

Singh et al. (2000) investigated the cancer chemopreventive capabilities of *Aloe vera* leaf pulp extract by analyzing Aloe's effects on the enzymes that play a role in carcinogen metabolism and those involved in cellular antioxidant activity. Random-bred Swiss albino male mice were divided into four groups: group 1 was fed a normal diet and sham treated with distilled water by oral gavage for 14 days; group 2 was also fed a normal diet; however, they were treated with 30 μl of *Aloe vera* leaf pulp extract per animal by oral gavage for 14 days; group 3 was also fed a normal diet; however, their treatment amount was 60 μl of *Aloe vera* leaf pulp extract per animal, also delivered by oral gavage; group 4 was fed a diet containing 0.75% butylated hydroxyanisole (BHA) for 14 days. The last group served as a positive control because BHA is known to be an anticarcinogenic compound. The animals were killed and their livers homogenized. The resulting supernatant from the livers was used to assay for total cytosolic glutathione *S*-transferase, DT-diaphorase, lactate dehydrogenase, and antioxidant enzymes. The pellet was used for assaying cytochrome P450, cytochrome *b*5, cytochrome P450 reductase, cytochrome *b*5 reductase, and lipid peroxidation.

During the treatment phase of the experiment there were no animal deaths or decreases in body weight. Additionally, there was no indication of cellular damage. *Aloe vera* leaf pulp decreased the levels of cytochromes P450 and *b*5, but increased the activities of cytochrome P450 and *b*5 reductases. There were significant increases in the activity of glutathione *S*-transferase and DT-diaphorase in the high-dose group as compared to the control. In the low-dose group, the enzyme activity was comparable with that of the control group. The activity of lactate dehydrogenase was decreased in a dose-dependent manner. The activities of the antioxidant enzymes (glutathione, glutathione peroxidase, glutathione reductase, superoxide dimutase, and catalase) were all increased in a dose-dependent manner. The extent of lipid peroxidation was decreased significantly in the high-dose group; the low-dose group was also inhibited but it was not significant (Singh et al. 2000).

Lee (2001) reported aloe-emodin–induced apoptosis in lung cell carcinoma cell lines CH27 (human lung squamous carcinoma cells) and H460 (human lung non–small cell carcinoma cells). During apoptosis increases in cytochrome *c* and activation of caspase-3 were observed and expression of protein kinase C appeared to occur downstream of caspase-3.

Kuo et al. (2002) reported that aloe-emodin inhibited cell proliferation and induced apoptosis in two liver cancer cell lines, Hep G2 and Hep 3B. Hep G2 cells induced p53 (tumor suppressor gene) expression and was accompanied by induction of p21 (cell cycle regulator) expression that was associated with a cell cycle arrest in G1 phase. Fas/APO 1 (type one I membrane protein) receptors and Bax (proapoptotic protein) expression was increased in Hep G2 with aloe-emodin. In Hep 3B cells, the inhibition of cell proliferation by aloe-emodin was mediated through p21, and neither cell cycle arrest nor an increase in the level of Fas/APO1 receptor was observed. Bax expression was increased by aloe emodin in Hep 3B cells.

Wasserman et al. (2002) described Merkel cell carcinoma (MCC) as a rare and aggressive tumor of the skin, also known as a primary neuroendocrine carcinoma of the skin. A free floating cell line was established from a metastasis from a MCC patient. Aloe-emodin decreased the viability of carcinoma cells after 72 h of treatment. The results are statistically significant ($p < .02$) for 10 μmol and higher concentrations (100 μmol) ($p < .001$). Aloin was also investigated at the same concentration range and no effect was detected.

Aloe arborescens

Yagi et al. (1977) injected aloemannan, a partially acetylated β-D-mannan isolated from the leaves of *Aloe arborescens,* intraperitoneally into ICR mice (10 animals/group) that were implanted with sarcoma-180 cells. Doses of 5 and 100 mg/kg were administered daily for 10 days and produced inhibition ratios of 38.1% and 48.1%, respectively. No toxicity was observed.

Imanishi et al. (1981) administered aloctin A, a glycoprotein of *Aloe arborescens*, intraperitoneally to BALB/c mice (5 to 6 weeks of age) once daily for 5 days after tumor implantation. Tumors were induced by methylcholanthrene. At 10 mg/kg/day for 5 days, aloctin A significantly inhibited the growth of the methylcholanthrene-induced fibrosarcomas. To determine if the aloctin A had direct cytotoxicity on tumor cells or was due to host-mediated effects, the authors tested the effect of aloctin A on various tumor cell lines. Aloctin A had almost no inhibitory effect on the growth of tumor cell lines up to a concentration of 200 μg/ml.

Uehara et al. (1996) measured the formation of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-DNA adducts by ^{32}P-postlabeling analysis. Cytochrome P450 (CYP) 1A1 and 1A2 protein levels were analyzed by ELISA (enzyme-linked immunosorbent assay) to assess the mechanism of chemoprevention of hepatocarcinogenesis by freeze-dried whole leaves of *Aloe arborescens*. Ten male F344 rats were fed a diet containing 30% *Aloe arborescens* over an 8-day period. The 11 control animals were fed a basal diet only. On day 7, all animals were subjected to a two-third partial hepatectomy (PH). Twelve hours after the PH, the rats received a single intragastric dose of the carcinogenic food pyrolysate (IQ) (100 mg/kg) to initiate hepatocarcinogenesis. Rats were killed 6, 12, 24, and 48 h after IQ administration.

The levels of adducts, expressed as relative adduct labeling values, in rats treated with *Aloe arborescens* were decreased as compared with the control group at hour 24 (36 h after PH) and had further decreases at hour 48. The levels of CYP1A2, known to be responsible for the activation of IQ, also were decreased at hour 48. The authors concluded that *Aloe arborescens* has the potential to decrease IQ-DNA adduct formation, presumably as a result of decreased formation of active metabolites (Uehara et al. 1996).

Shimpo et al. (2001) examined freeze-dried powder of whole-leaf *Aloe arborescens* Miller var. *natalensis* Berger (Japanese name Kidachi aloe) for its modifying effect on azoxymethane (AOM)-induced aberrant crypt foci (ACF), putative neoplastic lesions, in male F344 (4-week-old) rats. Rats were fed a basal diet (groups 1 and 5), experimental diet with 1% (groups 2, 3, and 6), or 5% (groups 4 and 7) aloe for 5 weeks. On days 7, 14, and 21, groups 1 to 4 were given a subcutaneous injection of 15 mg/kg of AOM and groups 5 to 7 received a subcutaneous injection of normal saline (4 ml/kg).

No rats died. The 5% aloe diet rats had a "mud-like" or soft feces. Body weight was reduced significantly in all the AOM-dosed rats compared to the AOM-untreated rats. ACF developed only in the rats treated with AOM (with or without aloe) and no ACF were present in the untreated rats. ACF were decreased by 35% and 14% with the 1% and 5% aloe treatments, respectively. AOM had no effect in the quinone reductase (QR) levels in the liver, although QR activity was significantly greater in the liver of rats fed 5% aloe with or without AOM treatment as compared to the rats fed a basal diet (Shimpo et al. 2001).

Furukawa et al. (2002) reported that freeze-dried whole leaf powder of *Aloe arborescens* modified the initiation phase of cancer in female Syrian hamsters (total = 90; group = 30). *N*-nitrosobis(2-oxopropyl)amine (BOP) was administered subcutaneously for 4 weeks at a dose of 10 mg/kg. Aloe was given in the diet at 0%, 1%, or 5% for 5 weeks. At 54 weeks all surviving animals were killed and the development of neoplastic and preneoplastic lesions was assessed histopathologically.

Aloe at 5% decreased significantly the number of animals with pancreatic adenocarcinomas (58.6%) compared to BOP alone (86.6%) and decreased significantly the number of animals with atypical hyperplasia (27.5%) compared to BOP alone (56.6%). Adenocarcinomas per animal were decreased significantly by Aloe at 5% (0.69 ± 0.66) compared to BOP alone (1.20 ± 0.76) and atypical hyperplasias per animal were decreased significantly (0.34 ± 0.61) compared to BOP alone (0.76 ± 0.77). The decreases seen in the group treated with Aloe at 1% were significant only for the number of animals with adenocarcinomas and the number of adenocarcinomas per animal, compared to BOP alone. The authors concluded that treatment with the *Aloe arborescens* derivative has an inhibitory effect during the induction phase for pancreatic neoplastic and preneoplastic lesions (Furukawa et al. 2002).

Shimpo et al. (2002) reported that the ethyl acetate extract of an acetone soluble *Aloe arborescens* Miller var. *natalensis* Berger (Japanese name Kidachi aloe) fraction inhibited 12-*O*-tetra-decanoylphorbol-13-acetate (TPA)-induced ear edema (an early marker of tumor promotion) in ICR mice (n: control = 4; TPA = 6; TPA + Aloe extract = 6). The effect of topical treatment of 1 mg of aloe with 1.6 nmol topically applied TPA to the ear was 40% reduction of edema ($p < .0005$). Aloe extract itself did not induce edema. The 20 mg of aloe extract together with 5 nmol TPA also inhibited the TPA-induced increase in epidermal putrescine level by 30% ($p < .005$). Aloe extract affected the promotion of skin papillomas by TPA in 7,12-dimethylbenz[a]anthracene (DMBA)-initiated mice ($n = 20$/group). The extract lowered the percentage of tumor-bearing mice by 45% after 10 weeks ($p < .005$) and average number of tumors decreased (no tumors in solvent control, 25.3 tumor in TPA-treated, and 7.7 tumors in aloe-treated mice) ($p < .001$).

PHOTOTOXICITY

Vath et al. (2002) exposed human skin fibroblast cell lines (ATCC CRL 1634) to 1 μM aloe-emodin and UVA radiation (320 to 400 nm) from two GE F40BL bulbs filtered through 3 mm of soft glass. The irradiance of this source was 2.9×10^{-3} W/cm^2. UVA exposure alone did not affect cell survival up to exposures of 5 J/cm^2. UVA-plus aloe-emodin reduced survival to 50% at around 3 J/cm^2. UVA plus aloe-emodin in D_2O (extends the lifetime of singlet oxygen) reduced survival to 50% at around 1 J/cm^2. The authors suggested that this finding supports a phototoxic mechanism that involves formation of singlet oxygen by aloe-emodin in the presence of UVA. Cells exposed to aloe-emodin in the dark had no effect on survivability. Aloe-emodin was shown to bind to DNA, although single strand breaks in plasmid (*E. coli*) DNA were not observed.

Vargas et al. (2002) exposed human red blood cells to aloe-emodin in phosphate-buffered saline (PBS) solution (20 to 80 μg/ml) and UVA-irradiated the cells under a Rayonet photochemical reactor equipped with 16 phosphor lamps (340 to 500 nm) or alternatively with an Osram HQL 250 Watt medium-pressure Hg lamp in a pyrex immersion-well photoreactor (radiation dose of 4.5 J/cm^2). Aloe-emodin caused cell lysis (>50%) within 70 min after irradiation of erythrocyte suspension. Hemolysis did not occur in the dark controls. Free radical scavengers reduced glutathione (GSH), and superoxide dismutase (SOD) had little protective effect on the photohemolytic process. Photohemolysis was decreased 20% by addition of serum proteins. Preirradiated aloe-emodin was responsible for 60% hemolysis during 80 min. When the photohemolysis tests were done under argon there was a 54% decrease in the effect. Photoperoxidation was observed in irradiated aloe-emodin/linoleic acid in PBS solution. Significant amounts of hydroperoxides were formed. When this test was performed under argon a 34% reduction in lipid peroxidation was seen.

The effect of 5×10^{-6} M aloe-emodin on acetylcholinesterase (ACE) activity on human erythrocyte membranes irradiated with UV light (340 to 500 nm) was determined. ACE activity was inhibited by 50% with respect to controls; other fractions were incubated with reactive oxygen scavengers butylated hydroxyanisole (BHA) (1 mM), sodium azide (1 mM), diazabicyclooctane (DABCO) (1 mM), and SOD (0.01 mg/ml). The ACE activity recovered by $34.1 \pm 5.4\%$, $21.0 \pm 4.8\%$, $21.9 \pm 3.8\%$, and $3.8 \pm 3.0\%$ (not significant), respectively. The authors thought the radical species and singlet oxygen production and subsequent peroxyl radical formation are involved in the photoinhibitory effects of aloe-emodin on ACE activity (Vargas et al. 2002).

Shiseido Safety Research Labs (2002) reported photoallergenicity of Aloe Arborescens CRS was evaluated by Adjuvant and Strip test method using female Hartley strain albino guinea pigs (five subjects per group). During the induction phase of the experiment, 0.1 ml of emulsified Freund's complete adjuvant (FCA) was prepared and injected intradermally at 4 corners of a clipped and shaved nuchal area of the subject. Then, 0.1 ml of 3% Aloe Arborescens CRS in distilled water was applied to the area and irradiated with 10.2 J/cm^2 of UVA. Similar application and irradiation continued for 4 days, and control groups were treated in the same manner, only using distilled water. During the challenge phase of the experiment (followed a 2-week resting period), 0.02 ml of Aloe Arborescens CRS—6%, 3%, 0.6%, and 0.3% in distilled water—were applied to a clipped and shaved flank symmetrically in duplicate. One side was irradiated with 10.2 J/cm^2 of UVA, and the other side was covered with aluminum foil to prevent exposure to the light. The reactions with respect to erythema, eschar formation, and edema were scored at 24, 48, and 72 h and followed a scoring system to evaluate.

It was concluded that Aloe Arborescens CRS does not possess photosensitizing potential under the test conditions since no reactions were observed in the animals of both the treated and control groups. One animal in the control group, however, was excluded from the estimation due to persistent light reaction (Shiseido Safety Research Labs 2002).

Shiseido Safety Research Labs (2002) reported on another study analyzing skin phototoxicity of Aloe Arborescens CRS in Hartley strain male albino guinea pigs. There were five subjects in each of the treatment and control groups. The test material for the treatment group was 100% and 3% Aloe Arborescens CRS in distilled water and for the control group it was 0.02% 8-methoxypsoralen (8-MOP) in ethanol. The dorsum of the subjects was shaved and depilated, and 24 h later, 0.02 ml of the test materials was applied to the hairless area symmetrically in duplicate. One side was irradiated with 14 J/cm^2 of UVA, and the other side was covered in aluminum foil to prevent exposure to the light. The skin reactions with respect to erythema and edema were evaluated at 24, 48, and 72 h after irradiation with a scoring system. There were no skin reactions for Aloe Arborescens CRS at the irradiated and unirradiated sites. However, the assessment score of 8-MOP (positive control) at concentration 0.02% was severely phototoxic. The Shiseido Safety Research Lab concluded that Aloe Arborescens CRS does not possess phototoxic potential under the test condition.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation/Sensitization

The Institut D'Expertise Clinique (2000) applied Aloe Barbadensis Leaf Water to the back of 10 healthy female adults. The test substance (0.02 ml) was applied to the back on a 50-mm^2 area of skin and occluded for 48 h. No reactions or pathological irritation were observed other than a very slight erythema in one person.

Photoallergy/Photosentization

Ivy Laboratories (1987a) applied a 0.1% Aloe extract (suntan product) to the lower back of 25 healthy Caucasian subjects with no history of sun sensitivity. Each subject underwent three phases: pretesting, induction, and challenge. In the pretesting phase the minimal erythema dose (MED) was established on the midback (1-cm diameter) in 25% increments from the xenon arc solar simulator (150-watt compact xenon arc source equipped with UV-reflecting dichroic mirror and 1-mm-thick Schott WG-320 filter to produce simulation of solar UVB spectrum 290 to 320 nm). The MED was the area with distinct erythema after 20 to 24 h.

The induction phase involved application of (10 μl/cm^2) of aloe extract to a 2 $\times$ 2-cm section of the lower back and covered for 24 h. The site was then wiped and exposed to the MED and left open for 24 h and occluded for another 24 h. The patches were removed and exposed to 3 MEDs of solar simulator radiation. This sequence was repeated for the same test sites twice weekly for 3 weeks. The challenge phase occurred 10 to 14 days after the last induction exposure. The aloe was reapplied to the same area and in the same manner but in duplicate to a newly designated skin site of the same dimensions on the opposite side of the lower back and occluded for 24 h. Each site was irradiated with 4 J/cm^2 of UVA (UVB was filtered from the solar simulator with a 1-mm-thick UG5 filter). The duplicate site was unirradiated; another skin site untreated with aloe was exposed to 4 J/cm^2 of UVA. No adverse reaction at any phase of the experiment was seen (Ivy Laboratories 1987a).

Ivy Laboratories (1987b) applied a suntan preparation containing 0.1% aloe extract to the lower back of 10 healthy, Caucasian adults with fair skin (skin type I: always burns easily, never tans; II: always burns easily, tans minimally; and III: burns moderately, tans gradually). All subjects were over the age of 18, with no medical or dermatological illness. Aloe (50 μl) was applied to a 2 $\times$ 2-cm area in duplicate, allowed to dry, and covered. Six hours later one site was exposed to 30 J/cm^2 of UVA radiation. An adjacent skin site was treated with vehicle (hydrophilic ointment USP) and exposed to the UVA. Reactions were graded immediately, and at 24 and 48 hours.

No abnormal reactions were observed. One subject had minimal erythema reaction immediately. One individual had a minimal erythema to the vehicle control and the aloe immediately that persisted at 24 h and cleared by 48 h (Ivy Laboratories 1987b).

Ivy Laboratories (1988) applied a 0.01% Aloe Barbadensis Flower Extract (suntan product) to the lower back of 25 healthy Caucasian subjects with no history of sun sensitivity. Each subject underwent three phases: pretesting, induction, and challenge. In the pretesting phase the minimal erythema dose (MED) was established on the midback (1-cm diameter) in 25% increments from the xenon arc solar simulator (150-watt compact xenon arc source equipped with UV-reflecting dichroic mirror and 1-mm-thick Schott WG-320 filter to produce simulation of

solar UVB spectrum 290 to 320 nm). The MED was the area with distinct erythema after 20 to 24 h.

The induction phase involved application of aloe extract, 10 μl/cm^2, to a 2 × 2-cm section of the lower back and covered for 24 h. The site was then wiped and exposed to the MED and left open for 24 h and occluded for another 24 h. The patches were removed and exposed to 3 MEDs of solar simulator radiation. This sequence was repeated for the same test sites twice weekly for 3 weeks. The challenge phase occurred 10 to 14 days after the last induction exposure. The aloe was reapplied to the same area and in the same manner but in duplicate to a newly designated skin site of the same dimensions on the opposite side of the lower back and occluded for 24 h. Each site was irradiated with 4 J/cm^2 of UVA (UVB was filtered from the solar simulator with a 1-mm-thick UG5 filter). The duplicate site was unirradiated; another skin site untreated with aloe was exposed to 4 J/cm^2 of UVA.

No adverse reactions were noted, except for mild to moderate erythema, scaling, and tanning (Ivy Laboratories 1988).

The Consumer Product Testing Co. (1996) applied a 0.1% Aloe extract (suntan product) to the lower back of 26 healthy Caucasian subjects with no history of sun sensitivity. Each subject underwent three phases: pretesting, induction, and challenge. One subject dropped out for personal reasons. In the pretesting phase the minimal erythema dose (MED) was established on the midback (1-cm diameter) in 25% increments from the xenon arc solar simulator (150-watt compact xenon arc source equipped with UV-reflecting dichroic mirror and 1-mm-thick Schott WG-320 filter to produce simulation of solar UVB spectrum 290 to 320 nm).

The induction phase involved application of aloe extract (0.2 g) to a 3/4 × 3/4-inch area of the lower back and covered for 24 h. The site was then wiped and exposed to the MED and left open for 24 h and occluded for another 24 h. The patches were removed and exposed to two times the MED as determined for each subject. This sequence was repeated for the same test sites twice weekly for 3 weeks. The challenge phase occurred 10 to 14 days after the last induction exposure. The product was reapplied to the same area and in the same manner but in duplicate to a new designated skin site of the same dimensions on the opposite side of the lower back and occluded for 24 h. Each site was irradiated for 3 min with UVA (UVB was filtered from the solar simulator with a 1-mm-thick UG5 filter). The duplicate site was unirradiated; another skin site untreated with the product was exposed to 4 J/cm^2 of UVA.

No adverse reactions at any phase of the experiment were observed (Consumer Product Testing Co. 1996).

Ivy Laboratories (1996) applied a 0.5% Aloe extract (other suntan product) to the lower back of 31 healthy Caucasian subjects with no history of sun sensitivity. Each subject underwent three phases: pretesting, induction, and challenge. In the pretesting phase the minimal erythema dose (MED) was established on the midback (1-cm diameter) in 25% increments from the xenon arc solar simulator (150-watt compact xenon arc source equipped with UV-reflecting dichroic mirror and 1-mm-thick Schott WG-320 filter to produce simulation of solar UVB spectrum 290 to 320 nm). The MED was the area with distinct erythema after 20 to 24 h.

The induction phase involved application of 80 mg of the product to a 2 × 2-cm area of the lower back and covered for 24 h. The site was then wiped and exposed to the MED and left open for 24 h and occluded for another 24 h. The patches were removed and exposed to 3 MEDs of solar simulator radiation. This sequence was repeated for the same test sites twice weekly for 3 weeks. The challenge phase occurred 10 to 14 days after the last induction exposure. The product was reapplied to the same area and in the same manner but in duplicate to a new designated skin site of the same dimensions on the opposite side of the lower back and occluded for 24 h. Each site was irradiated with 4 J/cm^2 of UVA (UVB was filtered from the solar simulator with a 1-mm-thick UG345 filter). The duplicate site was unirradiated; another skin site untreated with the product was exposed to 4 J/cm^2 of UVA.

A total of 25 subjects exhibited, at most, mild erythema, desquamation, and tanning at any phase of the experiment. Six subjects dropped out of the study for personal reasons (Ivy Laboratories 1996).

KGL Inc. (2000) applied a product containing 0.1% aloe extract to the lower back of 26 Caucasian adults with fair skin (skin type I: always burns easily, never tans; II: always burns easily, tans minimally; and III: burns moderately, tans gradually) over the age of 18 with no medical or dermatological illness and no history of sun sensitivity. The subjects underwent three phases: pretesting, induction, and challenge. In the pretesting phase the minimal erythema dose (MED) was established on the midback (1-cm diameter) in 25% increments from the xenon arc solar simulator (150-watt compact xenon arc source equipped with UV-reflecting dichroic mirror and 1-mm-thick Schott WG-320 filter to produce simulation of solar UVB spectrum 290 to 400 nm). The MED was the area with distinct erythema after 20 to 24 h.

The induction phase involved application of 40 mg of the product to a 2 × 2-cm area of the lower back and covered for 24 h. The site was then wiped and exposed to the MED and left open for 48 h and occluded for another 24 h. The patches were removed and exposed to 3 times the MED as determined for each subject. This sequence was repeated for the same test sites twice weekly for 3 weeks. The challenge phase occurred 12 days after the last induction exposure. The product was reapplied to the same area and in the same manner but in duplicate to a new designated skin site of the same dimensions on the opposite side of the lower back and occluded for 24 h. Each site was irradiated with 4 J/cm^2 of UVA (UVB was filtered from the solar simulator with a 1-mm-thick UG5 filter). The duplicate site was unirradiated; another skin site untreated with the product was exposed to 4 J/cm^2 of UVA.

No adverse reactions at any phase of the experiment were seen (KGL Inc. 2000).

KGL Inc. (2001) applied a product containing 0.5% aloe extract to the lower back of 26 healthy Caucasian subjects with no history of sun sensitivity. Subjects were of skin type I: always burns easily, never tans; II: always burns easily, tans minimally; and III: burns moderately, tans gradually and over the age of 18 with no medical or dermatological illness. The subjects underwent three phases: pretesting, induction, and challenge. One subject dropped out of the study for personal reasons. In the pretesting phase the minimal erythema dose (MED) was established on the midback (1-cm diameter) in 25% increments from the xenon arc solar simulator (150-watt compact xenon arc source equipped with UV-reflecting dichroic mirror and 1-mm-thick Schott WG-320 filter to produce simulation of solar UVB spectrum 290 to 320 nm). The MED was the area with distinct erythema after 20 to 24 h.

The induction phase involved application of 40 mg of aloe extract to a 2 × 2-cm section of the lower back and covered for 24 h. The site was then wiped and exposed to 3 MEDs and left open for 48 h and occluded for another 24 h. The patches were removed and exposed to 3 MEDs of solar simulator radiation. This sequence was repeated for the same test sites twice weekly for 3 weeks. The challenge phase occurred 16 days after the last induction exposure. The product was reapplied to the same area and in the same manner but in duplicate to a new designated skin site of the same dimensions on the opposite side of the lower back and occluded for 24 hours. Each site was irradiated with 4 J/cm^2 of UVA (UVB was filtered from the solar simulator with a 1-mm-thick UG345 filter). The duplicate site was unirradiated; another skin site untreated with the product was exposed to 4 J/cm^2 of UVA.

No adverse reactions at any phase of the experiment were seen (KGL Inc. 2001). Table 13 summarizes the results of these product studies.

In addition to the above studies, CTFA (2003) provided results of testing of a number of products containing different aloe-derived ingredients for photosensitization and phototoxicity on human volunteers. The subjects were categorized as having skin type I (always burns easily, never tans), skin type II (always burns easily, tans minimally), and skin type III (burns moderately, tans gradually) and the ultraviolet dosage was adjusted according to the skin type. Semioccluded patches of test materials were employed for each type of study. The compounds studied and results obtained are provided in Table 14.

Effects on Pregnancy

In the *CRC Handbook of Medicinal Herbs* (Duke 1985), *Aloe barbadensis* is cited as being considered abortive. Briggs (1995) mentions that aloe latex or juice should not be ingested during pregnancy due to the potent cathartic action of anthraquinones that may stimulate reflex contractions of the uterus, possibly resulting in premature labor or miscarriage; and nursing mothers should not take aloes as a laxative because the anthraquinones may be secreted into their milk, resulting in diarrhea in the baby.

Kacew (1993) reported that Aloe ingestion by the mother (from a laxative) increased intestinal peristalsis in the fetus, released meconium in uterus, and caused kidney damage in the fetus. In addition, Aloe administration to the infant through the breast milk caused increased bowel activity in the infant.

Treatment Uses

Asthma

Shida et al. (1985) reported that 33 patients received an Aloe extract (species not indicated) orally for 6 months for the treatment of adult bronchial asthma. The clinical trial was conducted for 24 weeks, preceded by a 4-week basal period. Five milliliters of a 20% solution of Aloe extract in saline was administered orally twice a day. No adverse effects were reported throughout the 24-week trial period.

Heart Disease

Agarwal (1985) treated 5000 patients with evidence of ischemic heart disease with 100 g of the fresh flesh gelatin of *Aloe vera* and 20 g of Husk of Isabgol. The two ingredients were mixed with wheat flour to make a bread and eaten daily at lunch and dinner for 5 years. The scope of this study was to

TABLE 13
Photoallergy and photosensitization studies containing aloe-derived ingredients

Product	Concentration of ingredient	No. of subjects	Results	Reference
Suntan product	0.1% Aloe extract	25	No adverse reaction observed	Ivy Laboratories 1987a
Suntan product	0.1% Aloe extract	10	No adverse reaction observed	Ivy Laboratories 1987b
Suntan product	0.01% Aloe Barbadensis Flower extract	25	No adverse reaction observed	Ivy Laboratories 1988
Suntan product	0.1% Aloe extract	26	No adverse reaction observed	Ivy Laboratories 1996
Suntan product	0.1% Aloe extract	25	No adverse reaction observed	KGL Inc. 2000
Other suntan preparations	0.5% Aloe extract	31	No adverse reaction observed	Ivy Laboratories 1996
Other suntan preparations	0.5% Aloe extract	26	No adverse reaction observed	KGL Inc. 2001

TABLE 14
Further photosensitization and phototoxicity studies containing aloe-derived ingredients (CTFA 2003)

Product and ingredient	Concentration	Type of study	No. of subjects	Results
Toner—*Aloe barbadensis* leaf extract	0.05%	Phototoxicity	10	No reactions on either irradiated or non-irradiated test material contact sites were observed. No dermal phototoxicity observed
		Dermal photoallergy	26	No dermal photoallergy or dermal sensitization in human subjects was observed
Lotion—*Aloe barbadensis* inner leaf gel concentrate	0.1%	Phototoxicity	10	No reactions on either irradiated or nonirradiated test material contact sites were observed. No dermal phototoxicity observed
		Dermal photoallergy	30	No dermal photoallergy or dermal sensitization in human subjects was observed
Hand lotion—Aloe extract Vera containing polysaccharide & no detectable anthraquinone	0.1%	Phototoxicity	10	No reactions on either irradiated or nonirradiated test material contact sites were observed. No dermal phototoxicity observed
		Dermal photoallergy	29	No dermal photoallergy or dermal sensitization in human subjects was observed
Lotion—*Aloe barbadensis* leaf extract	0.05%	Phototoxicity	10	No reactions on either irradiated or nonirradiated test material contact sites were observed. No dermal phototoxicity observed
		Dermal photoallergy	26	No dermal photoallergy or dermal sensitization in human subjects was observed
Powder—*Aloe vera* gel, powder with aloin levels 3–5 ppm in reconstituted product	0.01%	Phototoxicity	10	No reactions on either irradiated or nonirradiated test material contact sites were observed. No dermal phototoxicity observed
		Dermal photoallergy	28	No dermal photoallergy or dermal sensitization in human subjects was observed
Oil—*Aloe ferox* leaf extract	0.2%	Phototoxicity	10	No reactions on either irradiated or nonirradiated test material contact sites were observed. No dermal phototoxicity observed
		Dermal photoallergy	29	No dermal photoallergy or dermal sensitization in human subjects was observed
Gel—*Aloe barbadensis* leaf extract with anthraquinone less than 5 ppm	0.1%	Phototoxicity	10	No reactions on either irradiated or nonirradiated test material contact sites were observed. No dermal phototoxicity observed

TABLE 14
Further photosensitization and phototoxicity studies containing aloe-derived ingredients (CTFA 2003) *(Continued)*

Product and ingredient	Concentration	Type of study	No. of subjects	Results
		Dermal photoallergy	25	No dermal photoallergy or dermal sensitization in human subjects was observed
VP40:1 or biostatic Aloe Vera	*Aloe vera* gel 65% and Aloe concentrate 0.75%	Phototoxicity	10	No reactions on either irradiated or nonirradiated test material contact sites were observed. No dermal phototoxicity observed
		Dermal photoallergy	28	No dermal photoallergy or dermal sensitization in human subjects was observed
		Dermal photoallergy	30	No dermal photoallergy or dermal sensitization in human subjects was observed

observe the effects of the two ingredients in treating heart disease and associated complications. There were no adverse side effects noted in this study.

Diabetes

Ghannam et al. (1986) studied five otherwise healthy, non–insulin-dependent diabetic patients in a study of the antidiabetic activity of Aloes (see previous definition under miscellaneous biology). Apart from the blood sugar levels, all routine hematological and biochemical indices were normal. After 2 to 24 weeks without any treatment, the patients were weighed. Serum from the fasting patients was obtained for measurement of glucose (FSG) and insulin (FSI). After the patients had been taking Aloes for 4 to 14 weeks, FSG estimations were repeated in all patients and FSI values in three patients. The Aloes, in the form of dried resin, were taken with water by three patients and swallowed with food by two patients. The dose was half a teaspoon daily. The patients followed their normal diet throughout the study. The FSG levels of every patient fell during the consumption of the Aloes from a mean of 273 $\pm$ 25 (*SE*) to 151 $\pm$ 23 mg/dl, $p < .001$. The patients' body weights and serum insulin levels were unchanged. No patient had side effects, although all noted a bitter taste produced by the Aloes. The amount consumed was insufficient to cause abdominal cramps or diarrhea. No electrolyte disturbances were noted on routine screening.

Bunyapraphatsara et al. (1996) enrolled 49 men and 23 women in a study to evaluate the effect of *Aloe vera* juice on diabetes. The treatment group received one tablespoon full of *Aloe vera* juice twice a day and two tablets of glibenclamide (5 mg). The control group received a placebo (made to look and taste like the *Aloe vera* juice) and the two tablets of glibenclamide. The control group had stable levels of fasting blood glucose, cholesterol, and triglycerides. The treatment group had a significant decrease in blood sugar levels within 2 weeks and triglycerides within 4 weeks. The *Aloe vera* juice did not cause any adverse effect throughout the study.

Ulcers

Blitz et al. (1963) treated 12 patients diagnosed with peptic ulcers with 2 to 2.5 fluid drams of *Aloe vera* gel in a heavy liquid petrolatum emulsion (duration of treatment not specified). All patients recovered completely.

Atherton (1998) treated 7 patients with chronic leg ulcers with an *Aloe vera* gel orally (60 ml of fluid) and a topical Aloe jelly (amount not given). One patient withdrew due to the stinging sensation she felt when the Aloe jelly was applied; otherwise, none of the other patients had side effects. Three of the remaining six wounds healed completely, one healed partially and one showed no improvement. The six patients were pleased with the cleansing effect of the Aloe jelly reporting that there was less exudate and odor from their wounds. The author stated that swabs taken from the leg ulcers demonstrated that the Aloe jelly destroyed infecting bacteria, especially *Pseudomonas*.

Garnick et al. (1998) conducted a study in which 40 patients diagnosed with aphthous stomatitis ulcers of the oral mucosal surfaces were randomly assigned to receive either control gel (carrier gel), carrier gel with *Aloe vera*, carrier gel with silicon dioxide and *Aloe vera*, carrier gel with *Aloe vera* and allantoin, or carrier gel with silicon dioxide, *Aloe vera,* and allantoin. Each subject applied the gel product with a cotton swab and then brushed their teeth with the gel, spreading the gel throughout

the mouth. The subjects using the carrier gel with silicon dioxide, *Aloe vera*, and allantoin had significant decrease in lesion duration. There were no other significant results in the other groups in comparison with the control group.

Thomas et al. (1998) reported a study in which 30 subjects with pressure ulcers were randomized into two groups: a saline dressing control group and the acemannan (isolated from *Aloe barbadensis*) hydrogel experimental group. Acemannan hydrogel dressing was purchased commercially. No differences were observed in complete healing between the experimental and control groups. The authors concluded that acemannan hydrogel dressing is as effective as but not superior to a moist saline gauze dressing for pressure ulcer management.

Skin Disease

Zawahry et al. (1973) applied gauze soaked with gel that had been extracted from the center of *Aloe vera* leaves locally to chronic ulcers three to five times daily on three patients. All patients had chronic leg ulcers that had not responded to any other treatment. All three patients had an increase in the epithelialization of their ulcers. The only side effect reported was increased pain at the start of treatment; the pain diminished by the 2nd week. Additionally, the authors treated three patients with acne, three patients with seborrheic alopecia, and three patients with alopecia areata with the *Aloe vera* gel. All three ulcer patients showed improvement with their *Aloe vera* treatment. There was increased vascularization of all ulcers and removal of necrotic tissue. The three patients with seborrheic alopecia had some hair regrowth; the three acne patients had facial drying followed by improvement in their acne. Two of the three were acne free within 1 month. The three patients with alopecia areata had hair growth. None of the patients reported contact dermatitis or any type of irritation.

Syed et al. (1996) randomly allocated 60 psoriasis patients (36 males and 24 females) aged 18 to 50 years with slight to moderate chronic-type psoriasis to two parallel groups. A 0.5% *Aloe vera* preparation in a hydrophilic cream, using a combination of mineral oil and castor oil as vehicles, was provided to patients in the treatment group. A matching placebo cream that did not contain *Aloe vera* was given to the control group.

At the start and end of the study, full thickness 6-mm punch skin biopsies were taken under local anaesthesia from one lesion of each patient and were stained for the evaluation of acanthosis, parakeratosis, thinning, papillary vessel dilatation, and inflammatory infiltration. Patients were given a precoded 100-g tube (active or placebo) for 1 week of use. Additionally, patients were shown how to apply the trial preparation topically to their lesions three times daily (without occlusion and without exposure of lesions to sunlight) for 5 consecutive days, for a maximum of 15 applications per week. The study was restricted to four weeks of active treatment. To assess the clinical efficacy and tolerance of the trial preparation, patients were examined once a week for up to 16 weeks and thereafter followed up on a monthly basis for 8 months.

The trial preparation was well tolerated (all patients received 4 weeks of treatment) and no dropouts occurred. All 60 patients were available for efficacy analysis. During the 4-week trial period progressive decrease of desquamation, followed by decreased erythema and infiltration, resulting in moderate to excellent improvement or complete resolution of psoriatic lesions, was noted. By the end of the 4-week active treatment, 27/60 (45%) patients and 356/762 (46.7%) of psoriatic plaques were cleared. The *Aloe vera* cream cleared a significantly larger number of patients than the placebo (25/30 [83.3%] versus 2/30 [6.6%]). The *Aloe vera* group had a higher number of healed chronic plaques (328/396 [82.8%] versus 28/366 [7.7%]). Periodic laboratory tests, including blood cell count and urinalysis, were all within normal range. Biopsy analysis revealed that cured lesions had a decrease in the levels of epidermal acanthosis, parakeratosis, thinning, papillary vessel dilatation, and inflamation. Patients experienced no treatment-related adverse symptoms, either local or systemic, and there were no reports of hypersensitivity or dermatitis (Syed et al. 1996).

Skin Damage

Heck et al. (1981) randomly assigned 18 burn patients with second-degree burns to be treated with either *Aloe vera* cream or Silvadene ointment. Burn wounds were first debrided and then covered with gauze containing the treatment material. Patients were seen on an alternate day schedule for wound care and evaluation; cultures were taken of the wounds. None of the patients had any significant bacterial colonization. The *Aloe vera* cream group had a mean healing time of 13 days whereas the Silvadene group had a mean healing time of 16.15 days. The difference was not significant. No adverse reactions occurred during the duration of the study.

Fulton (1990) studied the effects of *Aloe vera* gel on wound healing in 18 full-face dermabrasion patients. Following dermabrasion, the abraded face was divided in half. One side was treated with the standard polyethylene oxide gel wound dressing. The other side was treated with polyethylene oxide gel dressing saturated with stabilized *Aloe vera* gel. The polyethylene oxide dressing provided an excellent matrix for the release of *Aloe vera* gel during the initial 5 days of wound healing. By 24 to 48 h there was dramatic vasoconstriction and accompanying decrease in edema on the *Aloe vera* gel–treated side. By the 3rd to 4th day, there was less exudate and crusting at the *Aloe vera* gel site, and by the 5th to 6th the reepithelialization at the *Aloe vera* gel site was complete. Overall, wound healing was approximately 72 h faster at the Aloe site.

Visuthikosol et al. (1995) treated 27 patients with recent burn injuries and no previous treatment using a treatment protocol that divided their burn area into two treatment sites. The distal part of the burn site was dressed with *Aloe vera* gel (85%) and the other site was treated with Vaseline gauze. The *Aloe vera* gel site was also wrapped with gauze. Both burn wound areas had their dressings changed twice daily. The *Aloe vera* gel–treated

site had a significantly quicker healing time when compared to the Vaseline-treated sites. Side effects from the *Aloe vera* gel–treated sites were minor, some discomfort and pain. An in-depth description of the side effects was not presented in the paper.

Williams et al. (1996) studied *Aloe barbadensis* gel for its effect on ionizing radiation–induced skin toxicity. An initial 194 patients, previously diagnosed with breast cancer and with a planned course of radiation therapy to the breast and/or chest wall, were randomized in a double-blind manner to receive either an *Aloe barbadensis* gel or a placebo gel. Following analysis of these initial patients, another 108 patients were randomized to another trial to receive either the *Aloe barbadensis* gel or no treatment. The *Aloe barbadensis* gel was 98% pure. It was obtained from decorticated leaves of *Aloe barbadensis* plants. An inert gel was added to provide a gel consistency and was used as the placebo. The study medication (*Aloe barbadensis* gel or placebo) was applied lightly to the treatment field twice daily starting within 3 days of initiation of the radiation. The planned target radiation dose, including boost, ranged from 45 to 60 Gy. Patients were to follow "usual" skin care precautions given at the start of radiation therapy. No other prophylactic creams/gels/lotions were to be topically applied to the radiation field. However, treatment of established radiation-induced dermatitis was allowed. Patients with pruritus and/or marked erythema were to use a 1% hydrocortisone cream two to three times daily. The patient's chest wall skin was examined at study initiation and at weekly intervals. Scoring was as follows: 0 = normal; 1 = mild erythema; 2 = marked erythema with or without dry desquamation; or 3 = moist desquamation and/or ulceration. Skin dermatitis scores were virtually identical on both treatment arms during both of the trials. The only toxicity noted was allergic reactions that occurred in three patients treated with *Aloe barbadensis* gel and one treated with the placebo.

Olsen et al. (2001) reported on 70 patients (male and female), randomized to receive either *Aloe vera* or no treatment, to examine *Aloe vera*'s (purchased commercially from a local supplier) ability to prevent skin reactions in patients undergoing radiation therapy. Patients in the *Aloe vera* group began therapy on the 1st day of radiation therapy. They were instructed to wash with a mild soap and to apply the gel liberally to the affected area following their radiation treatment. Patients were encouraged to reapply the gel throughout the day. *Aloe vera* did not significantly protect against radiation induced skin changes. In addition, no allergic contact dermatitis occurred in the *Aloe vera* group.

Genital Herpes

Syed et al. (1997) randomized 60 adult males into two groups for treatment of genital herpes. Groups received either *Aloe vera* extract (0.5% by weight in a hydrophillic cream) or placebo (hydrophillic cream without *Aloe vera*). Treatment involved application of preparation three times daily for 5 consecutive days. The *Aloe vera* extract group had significantly shorter healing times when compared to the placebo group (4.9 days versus 12 days) and a higher number of patients healed in the *Aloe vera* extract group compared to the placebo group (20/30 versus 2/30). No drug-related adverse symptoms were noted; however, five patients did experience nonobjective itching which resolved within 24 h.

Cancer

Lissoni et al. (1998) treated 50 patients with locally advanced or metastatic solid tumors, for whom no other effective standard therapy was available, with either melatonin or melatonin plus *Aloe vera*. Melatonin was administered orally daily at a dose of 20 mg/day, whereas *Aloe vera* was administered as a tincture (*Aloe vera* leaves and alcohol) at a dose of 1 ml twice a day (mornings and evenings). Patients treated with melatonin alone did not see any tumor regression. In the melatonin/*Aloe vera*–treated group 12/24 patients had their disease stabilized, whereas only 7/26 in the melatonin only group had stabilized disease. The melatonin/*Aloe vera*–treated group had significantly higher survival rates for 1 year when compared to the melatonin only group. Diarrhea occurred in 17% of the melatonin/*Aloe vera*–treated group and was limited to day 1.

Case Reports

Treatment Cases

Collins and Collins (1935) reported that roentgen dermatitis can be treated using whole Aloe leaf. Their patient, a woman aged 31 was suffering with severe roentgen dermatitis and did not respond to various ointments including boric acid and phenol in olive oil. However, 24 h after a 1-h application of fresh *Aloe vera* leaf extract, the sensation of itching and burning had entirely subsided. After 5 weeks of treatment, there was complete regeneration of the skin, new hair growth, complete restoration of sensation, and absence of scar.

Wright (1936) reported that a 60-year-old woman who had received prolonged x-ray treatment for eczema developed fissuring and ulceration of the palmar surface of the right forefinger and thumb. She was treated with *Aloe vera* ointment. She applied the macerated inner gel liberally and covered the affected area with waxed paper. Within 3 months the hand had completely healed. This author also reported that a doctor diagnosed with x-ray ulceration of the left hand was treated with the whole leaf of *Aloe vera* and *Aloe vera* ointment (preparation unknown). Within 3 days of starting the treatment there was a marked improvement in the appearance of the fingers and in 3 weeks the skin was virtually healed.

Loveman (1937) reported two cases of dermatitis that were cured by fresh aloe leaf extract. A man aged 40 had severe roentgen ray dermatitis involving the back of both hands. After some other methods of treatment failed, fresh whole leaf extract of *Aloe vera* was used on the large ulcer and aloe leaf ointment was employed on the rest of the hand. This patient did not show any response for 2.5 weeks, but the condition significantly improved after 5 weeks of application. After 8 months of treatment,

the entire ulcer had healed and the skin texture had improved. The slow response of this patient was attributed to the fact that only certain parts of the edge of the ulcer were capable of regenerating epithelium, therefore it takes a longer time for the healing to start. Another case involved a 46-year-old man suffering with severe dermatitis on both hands who was treated with fresh whole leaf ointment as well as ointment. This patient reported that the pain subsided within 24 to 36 h and the healing was complete within 3 months. The authors noted that in both the cases, fresh leaf extract proved to be more effective than the ointment. Further, the ointment proved fairly satisfactory in the treatment of small erosions, ulcers, and keratoses along with general improvement of the skin texture.

Mandeville (1939) treated a 54-year-old male diagnosed with squamous cell epithelioma of the right side of his tongue and floor of his mouth with radium therapy and external deep x-ray. The patient developed a large ulcer on the tongue and floor of the mouth (5 × 1.5 cm). For treatment the patient held within his mouth the jelly-like substance of fresh *Aloe vera* leaf for an average of 7 hours a day. Treatment was for 10 weeks; by the end of the 10th week the ulcer was completely healed.

Sauchak (1977) reported the case of a 40-year-old male who self treated a boil on his right forearm with aloe leaves (species not given). After treatment, the right forearm and hand reddened, major edema occurred, and a large blister appeared. By the next day, the patient had erythema and blisters on the anterior surface of his chest, left shoulder, forearm, and chin. The patient was treated with dexamethasone, pipolfen, and oxycort; within 17 days the patient had recovered.

Adverse Reaction Cases

Hamilton (1932) noted the case of a 49-year-old woman with varicose eczema of both legs for 15 years who ingested two herbal capsules over a period of 12 h. Within 24 h she had a rash over her body, swollen eyelids, the face, arms, legs, groin, area under the breasts, and front of chest were covered with minute vesicles. An acute eczema resulted. The substance in the capsules was identified as Curacao Aloe (Curacao Aloe Extract is synonymous with Aloe Barbadensis Leaf Extract).

Morrow et al. (1980) stated that hypersensitivity, manifested by generalized nummular eczematous and papular dermatitis, and presumably by contact urticaria developed in a 47-year-old man after 4 years of using oral and topical Aloe. Patch tests for Aloe were positive in the patient.

Shoji (1982) reported a 66-year-old man with a 20-day history of itchy dermatitis. The man was using a topical Aloe jelly for treatment. He had a history of allergic contact dermatitis to an anti-inflammatory analgesic plaster. Initial examination showed large, erythematous, eczematous plaques and papules over most of the shoulders, waist, abdomen, and extremities. A punch biopsy revealed crusting, spongiosis, and a mild edema with a moderate perivascular infiltrate of lymphocytes, histiocytes, and eosinophils. Topical application of the Aloe jelly preparation was stopped and the eruption was treated with 0.12% betamethasone 17-valerate ointment. A patch test was made 1 week later for the Aloe jelly (*Aloe arborescens*). At 48 and 96 h the Aloe jelly had a positive result. Eight subjects functioned as the control for the patch test and they all had negative results for *Aloe arborescens*.

Nakamura and Kotajima (1984) reported on a 7-year-old boy who had no personal or family history of contact dermatitis, eczema, asthma, or hay fever. Four days after using the leaf jelly *Aloe arborescens* on his perioral region because of a scaly eruption, he had an itchy, erythematous, burning, papular, edematous eruption around the mouth. A patch test with the fresh leaf jelly was positive after 48 h. Six control subjects were all negative. The boy made a rapid recovery following the use of topical corticosteroids.

Hogan (1988) reported that after 3 years of stasis dermatitis involving the right ankle, a 66-year-old woman began treatment with jelly from an *Aloe vera* plant. A moderately erythematous, scaly, papular eruption developed in the "V" of her neck , on her arms, and on the right ankle. Prominent varicose veins were seen in the lower right leg. She was advised to stop using the jelly and to apply 0.1% amcinonide cream twice daily to the affected areas for 2 weeks. A patch test performed with *Aloe vera* jelly gave a strong reaction at 48 and 96 h.

Hunter and Frumkin (1991) reported the case of a 65-year-old woman who underwent a perioral chemical peel with standard Baker's solution without taping. The treated area healed well and the face looked pink but had no crusting or secretions. The patient thought she could improve the results further by applying *Aloe vera*. She cut the leaf of the plant, squeezed the juice, and applied it to her nasolabial folds. She felt an immediate sensation of burning and the area around the nasolabial folds became red and swollen. The patient was immediately started on oral diphenhydramine and a 1% hydrocortisone cream. A severe erythema occurred and crusting returned, which took approximately 10 weeks to resolve. After 5 months, the dermatitis had completely resolved.

Adverse Reaction Reports

In 1998, 27 adverse events due to *Aloe vera* were reported to the Food and Drug Administration (FDA) Special Nutritionals Adverse Event Monitoring System (FDA 1998). Most products that caused adverse events contained ingredients listed either as *Aloe vera* or *Aloe vera* extract. Events that were reported where *Aloe vera* was listed as the main ingredient were stomach problems, depression, low thyroid, attempted suicide, tender and sore kidneys, blackout, faint feeling, stroke, death, tiredness, dizziness, buzzing or tingling in the ears, red itchy rash, aseptic meningitis, and severe headache. Other events occurred; however, *Aloe vera* was not listed as the main ingredient. Some of those events were liver failure, kidney failure, death, seizure and stroke, nervousness, panic attacks, inability to concentrate, and ventricular fibrillation.

SUMMARY

Plant material derived from the Aloe plant is characterized by the plant species, the plant part, the material derived, and the constituents in that material. The materials currently described as cosmetic ingredients include Aloe Andongensis Extract, Aloe Andongensis Leaf Juice, Aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Arborescens Leaf Protoplasts, Aloe Barbadensis Flower Extract, Aloe Barbadensis Leaf, Aloe Barbadensis Leaf Extract, Aloe Barbadensis Leaf Juice, Aloe Barbadensis Leaf Polysaccharides, Aloe Barbadensis Leaf Water, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, and Aloe Ferox Leaf Juice Extract. These ingredients function primarily as skin conditioning agents and are included in cosmetics only at low concentrations.

The leaf of Aloe plants consists of two main parts: the pericyclic cells, found just below the plant's skin, and the inner central area of the leaf, i.e., the gel, which is used for cosmetic products. The pericyclic cells produce a bitter-yellow latex containing a number of anthraquinones, including emodin, a phototoxic compound that is also a gastrointestinal irritant responsible for cathartic effects. The gel contains polysaccharides. There are generally more than one polysaccharide found in the gel; they can be acetylated, partially acetylated, or not acetylated. An industry established limit for anthraquinones in aloe-derived material for nonmedicinal use is 50 ppm or lower.

Processing the Aloe leaf may be done in two ways. In both processes, the whole leaf is cut and quickly transported to the processing center. After washing, the whole leaves are either ground through a hammer mill and directed into a heating vat, or filleted to remove the inner gel. Heating is done to reduce the amount of slime and to stabilize the gel material. Filtration, addition of activated charcoal, and dialysis may be used to further clarify the material and remove anthraquinones that may be present.

The terminology used by industry in reporting the type of Aloe-derived material used in cosmetic products does not match the current terminology used to describe individual Aloe-derived cosmetic ingredients. Nonetheless, it is clear that Aloe-derived ingredients are reported to be used in a wide variety of cosmetic product types. Aloe-derived ingredients generally contain concentrations of raw material that are 0.1% or less, although several uses report raw material concentrations around 5%. The concentration of Aloe in the raw material also may vary from 100% to a low of 0.0005%.

No data were available on the dermal absorption of Aloe-derived ingredients. Oral administration of various anthraquinone components results in a rise in blood concentrations, wide systemic distribution, accumulation in the liver and kidneys, and excretion in urine and feces. Intravenous injection or oral administration of aloemannan (a polysaccharide component) resulted in systemic distribution and metabolism into smaller molecules. Intravenous injection resulted in excretion primarily in urine over the first 24 h, whereas oral administration resulted in excretion in the feces at levels higher than in urine over the first 24 h.

Aloe-derived material has fungicidal, antimicrobial, and antiviral activities, and has been effective in wound healing and infection treatment in animals. Polysaccharide material derived from the inner gel is not cytotoxic, but the latex material is cytotoxic. Glutathione peroxidase and superoxide dismutase activities are detected in Aloe-derived material. Other components have been shown to degrade bradykinin; increase levels of cyclin E, CDK2, and CDC 25A; stimulate TNF-α, IL-1β, and IL-6; reduce IL-10 production; inhibit tyrosinase; and suppress both contact hypersensitivity and delayed-type hypersensitivity.

Aloe barbadensis–derived ingredients (also known as *Aloe vera*) were not toxic in acute oral studies using mice (at doses up to 3 g/kg) and rats (dose not given). In parenteral studies, the LD_{50} using mice was >200 mg/kg, using rats was >50 mg/kg, and using dogs was >50 mg/kg. In intravenous studies the LD_{50} using mice was >80 mg/kg, using rats was >15 mg/kg, and using dogs was >10 mg/kg.

The 14-day NOEL for the Aloe polysaccharide, acemannan, in the diet of Sprague-Dawley rats, was 50,000 ppm or 4.1 to 4.6 g/kg day^{-1}. No significant toxicity was seen in mice, rats, or dogs given acemannan intravenously or intraperitoneally at 4-day intervals over 30 days, at maximum dose levels of 200, 50, and 50 mg/kg, respectively.

In a 3-month study using mice, *Aloe vera* (extracted in ethanol) given orally in drinking water at 100 mg/kg produced reproductive toxicity, inflammation, and mortality above that seen in control animals. Acemannan, at doses up to 1500 mg/kg day^{-1} in the diet of dogs and at doses up to 2000 mg/kg in the diet of mice for 180 days, had no observable effect.

Filtered and unfiltered *Aloe barbadensis*–derived material, fed to SPF Fischer 344 male rats for 1.5 or 5.5 months at 1% and 10%, with and without charcoal filtration (a common step in producing cosmetic ingredients), in a rat chow diet resulted in severe diarrhea and reduced growth rates for the unfiltered 10% preparation, such that this arm of the study was terminated. The filtered 10% and the unfiltered and filtered 1% preparation resulted in no adverse effects.

Aloe Ferox Leaf Extract instilled into the eye of male New Zealand white rabbits produced no significant changes, and the small changes that were seen at 1 h post instillation were reduced still further after 1 day, and not present at 2 days.

Aloe vera, extracted in methanol and given to mice at 100 mg/kg in drinking water for 3 months, caused significant sperm damage compared to controls. *Aloe barbadensis* extracted with water and given to pregnant Charles Foster albino rats on gestational days (GDs) 0 through 9 was an abortifacient and produced skeletal abnormalities.

Both negative and positive results were found in bacterial and mammalian cell genotoxicity assays using *Aloe barbadensis*–derived material, Aloe Ferox–derived material, and various anthraquinones derived from Aloe.

Aloin, a specific anthraquinone derived from Aloe, did not produce tumors when included in the feed of mice for 20 weeks, nor did aloin increase the incidence of colorectal tumors induced with 1,2-dimethylhydrazine. Aloe-emodin, given to mice in which neuroectodermal tumor cells, Ewing's sarcoma cells, and neuroblastoma cells had been injected, inhibited growth of malignant tumors. If treatment with Aloe-emodin was withheld until palpable tumors developed, tumor growth was inhibited.

Acemannan given to dogs and cats with malignant tumors that had failed conventional treatment produced clinical improvement in 12/43 animals with no signs of toxicity from the acemannan. Acemannan given to female CFW mice previously injected with murine sarcoma cells increased survival of the mice compared to controls. Acemannan given to cats diagnosed with feline leukemia resulted in survival in 29/41 animals at the end of 12 weeks. Fibrosarcomas in dogs and cats, treated with acemannan weekly for 6 weeks, followed by monthly injections for 1 year, surgical intervention, and radiation therapy, responded to the treatment with 7/13 survivors with a mean survival time of 240 days. *Aloe vera* gel injections decreased the growth of ascite hepatoma cells injected into the pleural space of male Fischer rats compared to controls. *Aloe vera* gel in the drinking water of Sprague-Dawley rats reduced the severity of diethylnitrosamine and 2-acetylaminofluorene induced liver cancers. The growth of implanted sarcoma 180 cells in mice was inhibited by aloemannan injected intraperitoneally.

Various in vitro assays, including DNA adduct formation, neuroblastoma cells in culture, lung carcinoma cells in culture, liver cancer cells in culture, and neuroendocrine skin carcinoma cells in culture demonstrated anticarcinogenic activity of aloe-emodin.

Freeze-dried whole leaves of *Aloe arborescens* fed to male F344 rats, which were then given a partial hepatectomy and an intragastric dose of a carcinogenic food pyrolysate, produced a decrease in DNA adducts. Freeze-dried whole leaves of *Aloe arborescens* fed to male F344 rats, which were then given azoxymethane, produced a reduced incidence of aberrant crypt foci. Freeze-dried whole leaves of *Aloe arborescens* fed to male F344 rats, which were given *N*-nitroso-bis(2-oxopropyl)amine, produced decreased pancreatic adenocarcinomas, pancreatic atypical hyperplasias, and total atypical hyperplasias. An ethyl acetate extract of *Aloe arborescens* applied topically to the ear of mice treated with 12-*O*-tetradecanoylphorbol-13-acetate reduced ear edema, an early marker for tumor production.

Aloe-emodin, another anthraquinone derived from Aloe, applied to the skin of mice that were subsequently treated with 15 J/m^2 UVB radiation three times weekly for up to 31 weeks, produced skin tumors in 16/20 mice. Although this was not significantly different from the UVB radiation alone group, there was an increase in the proportion of melanomas with Aloe-emodin and UVB radiation compared to UVB radiation alone. Aloe-emodin–treated human skin fibroblast cell lines, exposed to UVA radiation, had reduced survival compared to UVA radiation treatment alone. Aloe-emodin plus UVA radiation caused cell lysis in human red blood cells at a higher frequency than either aloe-emodin or UVA radiation alone. Free radical scavengers had little effect on the photochemical hemolytic effect. Aloe-emodin plus UVA radiation inhibited acetylcholinesterase activity in human erythrocyte membranes.

Aloe Barbadensis Leaf Water was not irritating when applied to the back of 10 healthy female adults. A suntan product with 0.1% Aloe extract applied to the back of 25 Caucasian subjects in areas that were subsequently given MED and 3 $\times$ MED doses of UVB radiation or in other areas that received 4 J/cm^2 UVA radiation produced no additive effect to the UVB or UVA radiation. Similar studies with products containing 0.01% Aloe Barbadensis Flower Extract and up to 0.5% Aloe extract produced similar results.

Therapeutic uses of Aloe-derived ingredients have included asthma, ischemic heart disease, diabetes, ulcers, skin disease, and cancer. In no case were adverse effects, other than diarrhea, noted.

Case reports include acute eczema, contact urticaria, and dermatitis in individuals who applied Aloe-derived ingredients topically.

A total of 27 adverse events were reported to FDA as a result of ingestion of products containing *Aloe vera* or *Aloe vera* extract.

DISCUSSION

The CIR Expert Panel recognized that aloe-derived ingredients are a mixture of polysaccharides, proteins, and other compounds including anthraquinones. Of significant concern were the anthraquinones identified in aloe-derived ingredients that have been shown to be toxicants. The Panel considered the available data on the several Aloe Barbadensis extracts and found that anthraquinone levels are well understood and can conform to the industry-established level of 50 ppm. Although the phototoxicity of aloe-emodin, a well-characterized anthraquinone component of Aloe plants, has been demonstrated, several clinical studies of preparations derived from *Aloe barbadensis* plants demonstrated no phototoxicity, confirming that the concentrations of aloe-emodin, or other anthraquinones, in such preparations are too low to induce phototoxicity.

The mixed results of genotoxicity tests using bacterial and mammalian cells indicated that aloe-emodin was most likely linked to positive findings, where they occurred. In animal tests, aloe-emodin was not geneotoxic. Chemicals identified as Aloe vera gel, Aloe vera whole leaf extract, and charcoal filtered Aloe vera whole leaf extract, are planned for study by the National Toxicology Program in both a carcinogenicity and photocarc.

The characterization of aloe-derived ingredients from other species was not clear, especially with highly productive whole leaf processing. Therefore, the Panel considered the available data insufficient to support the safety of these aloe-derived ingredients. In the absence of well-characterized derivatives, biological studies of these materials were considered necessary.

The studies needed are 28-day dermal toxicity studies on Aloe Andongensis Extract, Aloe Andongensis Leaf Juice, Aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, and Aloe Ferox Leaf Juice Extract (ingredients should be tested at current use concentrations). Such data may be developed on one ingredient from each species if it can be demonstrated that the tested material is representative of the group. In aloe-derived ingredients used in cosmetics, regardless of species, anthraquinone levels should not exceed 50 ppm.

The Panel indicated that data indicating the presence or absence of pesticide residues in Aloe plants harvested are limited. The Panel advised the industry that the total polychlorobiphenyl (PCB)/pesticide contamination of any plant-derived cosmetic ingredient should be limited to not more than 40 ppm, with not more than 10 ppm for any specific residue. The Panel also advised that limits were appropriate for the following impurities: arsenic (3 mg/kg maximum), heavy metals (20 mg/kg maximum), and lead (5 mg/kg maximum).

It was noted that inhalation exposure was of concern given the lack of inhalation toxicity findings in the report. The few ingredients used in aerosol formulations, however, are at very low concentrations. In addition, the Panel expects that the aerosolized cosmetic formulations in which these ingredients are found would not contain aerosolized particles of a size that are respirable.

CONCLUSION

The CIR Expert Panel concluded that Aloe Barbadensis Flower Extract, Aloe Barbadensis Leaf, Aloe Barbadensis Leaf Extract, Aloe Barbadensis Leaf Juice, Aloe Barbadensis Polysaccharides, and Aloe Barbadensis Leaf Water are safe as cosmetic ingredients in the practices of use and concentrations as described in this safety assessment, if anthraquinone levels in the ingredients do not exceed 50 ppm. The available data are insufficient to support the safety of Aloe Andongensis Extract, Aloe Andongensis Leaf Juice, Aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, or Aloe Ferox Leaf Juice Extract in cosmetic products.

REFERENCES

Active Organics, Inc. 2002. Material Safety Data Sheet. Actiphyte® of Aloe Vera 10 Fold. www.activeorganics.com

Agarwal, O. P. 1985. Prevention of atheromatous disease. *Angiology* 36:485–492.

Agarwala, O. M. 1997. Whole Leaf aloe gel vs. standard Aloe gel. *Drugs and Cosmeic Industry* Februayy 22–28.

Ajabnoor, M. A. 1990. Effects of aloes on blood glucose levels in normal and alloxan diabetic mice. *J Ethnopharmacol*. 28:215–220.

Ali, M. I., N. M. Shalaby, M. H. Elgamal, et al. 1999. Antifungal effects of different plant extracts and their major components of selected aloe species. *Phytother Res*. 13:401–407.

Aloecorp. 2001. Time, TEMPERATURE AND Sanitation. www.aloecorp.com

Arosio, B., N. Gagliano, L. M. Fusaro, et al. 2000. Aloe-emodin quinone pretreatment reduces acute liver injury induced by carbon tetrachloride. *Pharmacol Toxicol*. 87:229–233.

Atherton, P. 1998. *Aloe vera:* Magic or medicine. *Nurs Stand*. 12:49–54.

Avila, H., J. Rivero, F. Herrera, and G. Fraile. 1997. Cytotoxicity of a low molecular weight fraction from *Aloe vera* (*Aloe barbadensis* Miller) gel. *Toxicon*. 35:1423–1430.

Bader, S., L. Carineli, R. Cozzi, and O. Cozzoli. 1981. Natural hydroxyanthracenic polyglycosides as sunscreens. *Cosmet. Toilet*. 96:67–74.

Badria, F. A. 1994. Is man helpless against cancer? An environmental approach: antimutagenic agents from Egyptian food and medicinal preparations. *Cancer Lett*. 84:1–5.

Blitz, J., J. W. Smith, and J. R. Gerard. 1963. *Aloe vera* gel in peptic ulcer therapy: Preliminary report. *J. Am. Ost. Assoc*. 62:731–735.

Bobin M. F., M. Raymond, and M. C. Martin. 1994. UVA/UVB absorption properties of natural products. *Cosmet. Toilet*. 109:63–70.

Bouthet, C. F., V. R. Schirf, and W. D. Winters. 1995. Stimulation of neuron like cell growth by aloe substances. *Phytother Res*. 9:185–188.

Brasher, W. J., E. R. Zimmermann, and C. K. Collings. 1969. Effects of prednisolone, indomethacin and *Aloe vera* gel on tissue culture cells. *Oral Surg. Oral Med Oral Pathol*. 27:122–128.

Briggs, C. 1995. Herbal medicine: Aloe. *Can. Pharmaceut. J.* 128:48–50.

Brown, J. P., and P. S. Dietrich. 1979. Mutagenicity of anthraquinone and benzanthrone derivatives in the *Salmonella*/microsome test: Activation of anthraquinone glycosides by enzymic extracts of rat cecal bacteria.*Mutat. Res*. 66:9–24.

Brown, J. P., P. S. Dietrich, and R. J. Brown. 1977. Frameshift mutagenicity of certain naturally occurring phenolic compounds in the *Salmonella*/Microsome test: Activation of anthraquinone and flavonol glycosides by gut bacterial enzymes. *Biochem. Soc. Trans*. 4:1489–1492.

Budavari, S., ed. 1989. *The Merck Index. An encyclopedia of chemicals, drugs, and biologicals,* 11th ed. Rahway, NJ: Merck & Co.

Bunyapraphatsara, N., S. Yongchaiyudha, V. Rungpitarangsi, O. Chokechaijaroenporn. 1996. Anti-diabetic activity of *Aloe vera* juice. Clinical trial in diabetes mellitus patients in combination with glibenclamide. *Phytomedicine* 3:245–248.

Byeon, S. W., R. P. Pelley, S. E. Ullrich, et al. 1998. *Aloe barbadensis* extracts reduce the production of interleukin-10 after exposure to ultraviolet radiation. *J. Invest. Dermatol*. 110:811–817.

Carrington Labs. 2001. Product specifications for aloe. www.carringtonlabs.com

Capasso, F., F. Borrelli, R. Capasso, et al. 1998. Aloe and its therapeutic use. *Phytother. Res*. 12:S124–127.

Capasso, F., N. Mascolo, G. Autore, and M. R. Duraccio. 1983. Effect of Indomethacin on Aloin and 1,8 Dioanthraquinone-induced production of prostaglandin in rat isolated colon. *Prostaglandin* 26:557–562.

Chithra, P., G. B. Sajithlal, and G. Chandrakasan. 1998. Influence of Aloe vera on collagen charactersitics in healing dermal wounds in rats. *Mol. Cell. Biochem*. 181:71–76.

Collin C. E., and C. Collins. 1935. Roentgen dermatitis treated with fresh whole leaf of *Aloe vera*. *Am. J. Roentgen*. 33:396–397.

Consumer Product Testing Co. 1996. Photoallergy test procedure: Aloe extract 0.1% in product type 77. Unpublished data. 9 pages.[2]

Corsi, M. M., A. A. Bertelli, G. Gaja, et al. 1998. The therapeutic potential of *Aloe vera* in tumor bearing rats. *Int. J. Tissue React*. 20:115–118.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2002. Determination of Aloe-emodin in Aloe Arborescens CRS. Unpublished data submitted by CTFA. 2 pages.[2]

CTFA. 2003. Photosensitization and phototoxicity studies of products containing Aloe-derived ingredients. Unpublished data. 233 pages.[2]

Danhof, I. E., and W. McAnalley. 1983. Stabilized *Aloe vera*: Effect on human skin cells. *Drug and Cosmetic Industry* 133, 52, 54, 105–106.

[2]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

Danhof, I. E. 1998. Position statement on polysaccharides. http://www.gothica.com.iasc.poly.html.

Davis, R. H., J. J. Di Donato, G. M. Hartman, and R. C. Haas. 1994a. Anti-inflammatory and wound healing of growth substances in *Aloe vera*. *J. Am. Pediatr. Med. Assoc.* 84:77–81.

Davis, R. H., J. J. DiDonato, R. W. S. Johnson, and C. B. Stewart. 1994b. *Aloe vera*, hydrocortisone and sterol influence on wound tensile strength and anti-inflammation. *J. Am. Pediatr. Med. Assoc.* 84:614–621.

de Witte, P. 1993. Metabolism and pharmacokinetics of anthranoids. *Pharmacology*. 47(suppl.1):86–97.

Dhar, M. L., B. N. Dhar, B. N. Dhawah, et al. 1968. Screening of Indian plants for biological activity Part 1. *Ind. J. Exp. Biol.* 6:232–247.

Diehl, B., and E. E. Teichmuller. 1998. *Aloe vera*, quality inspection and identification. *Agro-Food Industry Hi-tech*. 9:14–16.

Dixit, V. P., and S. Joshi. 1983. Effect of *Aloe barbadensis* and clofibrate on serum lipids in Triton-induced hyperlipidaemia in Presbytis monkeys. *Ind. J. Med. Res.* 78:417–421.

Duke, J. A. eds. 1985. Aloe barbadensis *Mill. CRC handbook of medicinal herbs,* 31–32. Boca Raton, FL: CRC Press.

Duke, J. A., and S. M. Beckstrom-Sternberg. 1994. "Acceptable" levels of flavoring ingredients? *Dev. Food Sci.* 34:741–757.

Egger, S. F., G. S. Brown, L. S. Kelsey, et al. 1996. Hematopoietic augmentation by a β-(1,4)-linked mannan. *Cancer Immunol. Immunother.* 43:195–205.

European Union 2000. Opinion concerning the first update of the inventory of ingredients employed in cosmetic products. Section II: Perfume and aromatic raw materials. http://europa.eu.int/comm/food/fs/sc/sccp/out131_en.pdf

European Commission. 2002. The rules governing cosmetic products in the European Union. Volume 1.Cosmetic Legislation—Cosmetic products. http://dg3.eudra.org/F3/home.html.

Esteban, A., J. M. Zapata, L. Casano, M. Martin, and B. Sabater. 2000. Peroxidase activity in *Aloe barbadensis* commercial gel: Probable role in skin protection.*Planta Med.* 66:724–727.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. *FDA Database*. Washington, DC: FDA.

FDA. 1992. Modification in voluntary filing of cosmetic product ingredient and cosmetic raw composition statements. Final rule. *Fed. Register* 57:3128–3130.

FDA. 1998. The Special Nutritionals/Adverse Event Monitoring System Web Report for aloe. http://www.cfsan.fda.gov.

FDA. 2001. Frequency of use of cosmetic ingredients. *FDA Database.* Washington, DC: FDA.

Fogelman, R. W., J. M. Chapdelaine, R. H. Carpenter, and B. H. McAnalley. 1992a. Toxicologic evaluation of injectable acemannan in the mouse, rat and dog. *Vet. Hum. Toxicol.* 34:201–205.

Fogleman, R. W., T. E. Sheelenberger, M. F. Balmer, et al. 1992b. Subchronic Oral administration of acemannan in the rat and dog. *Vet. Hum. Toxicol.* 34:144–147.

Fujita, K., R. Teradaira, and T. Nagatsu. 1976. Bradykinase activity of aloe extract.*Biochem. Pharmacol.* 25:205.

Fujita, K., Y. Yamada, K. Azuma, and S. Hirozawa. 1978. Effect of leaf extracts of *Aloe arborescens* Mill subsp *natalensis* Berger on growth of *Trichophyton mentagrophytes*. *Antimicrob. Agents Chemother.* 14:132–136.

Fulton, J. E. 1990. The stimulation of postdermabrasion wound healing with stabilized *Aloe vera* gel–polyethylene oxide dressing. *J. Dermatol. Surg. Oncol.* 16:460–467.

Furukawa F., A. Nishikawa, T. Chihara, K. Shimpo, H. Beppu, H. Kuzuya, I. S. Lee, and M. Hirose. 2002. Chemopreventive effects of *Aloe arborescens* on *N*-nitrobis(2-oxypropyl)amine-induced pancreatic carcinogenesis. *Cancer Lett.* 25:117–122.

Garnick, J. J., B. Singh, and G. Winkley. 1998. Effectiveness of a medicament containing silicon dioxide, aloe, and allantoin on aphthous stomatitis. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Oral Endod.* 86:550–556.

Ghannam, N., M. Kingston, I. A. Al-Meshaal, M. Tariq, N. S. Parman, and N. Woodhouse. 1986. The antidiabetic activity of aloes: Preliminary clinical and experimental observations. *Horm. Res.* 24:288–294.

Gjerstad, G. 1969. An appraisal of the *Aloe vera* juice. *Am. Perfum. Cosmet.* 84:43–46.

Gottschalck, T. E., and G. N. McEwen, Jr., eds. 2004. *International cosmetic ingredient dictionary and handbook,* 10th ed., Vol. 1, 76–81. Washington, DC: CTFA.

Gowda, D. C., B. Neelisiddaiah, and V. Anjaneyalu. 1979. Structural studies of polysaccharides from *Aloe vera*. *Carbohydr. Res.*72:201–205.

Gramatica, P. D. Monti, G. Speranza, and P. Manitto, 1982. Aloe revisited, the structure of Aloeresin A. *Tetrahedron Lett.* 23:2423–2424.

Green, M. W. 1941. The irritant effect of Aloin. Preliminary note. *J. Am. Pharmaceut. Assoc.* 30:186–187.

Grindlay, D., and T. Reynolds. 1986. The *Aloe vera* phenomenon: A review of the properties and modern uses of the leaf parenchyma gel. *J. Ethnopharmacol.* 16:117–151.

Gutterman, Y., and E. Chauser-Volfson. 2000. The distribution of the phenolic metabolites barbaloin, aloeresin and aloenin as a peripheral defense strategy in the succulent leaf parts of *Aloe arborescens*. *Biochem. Syst. Ecol.* 28:825–838.

Hamilton, I. 1932. Acute vesicular dermatitis caused by curacao aloes. *Med. J.Aust.*. 1:302–303.

Hammer Pharma. 2000. Hamma Pharma S.p.A. Herbal extracts. Aloe dry extract. http://www.hammerpharma.it/specifications/1/htm.

Harris, C., K. Pierce, G. King, et al. 1991. Efficacy of acemannan in treatment of canine and feline spontaneous neoplasms. *Mol. Biother.* 3:207–213.

Hay, J. E., and L. J. Haynes. 1956. The aloins. Part I. The structure of barbaloin. *J. Chem. Soc.* 3141–3147.

Heck, E., M. Head, D. Nowak, et al. 1981. *Aloe vera* (gel) cream as a topical treatment for outpatient burns. *Burns* 7:291–294.

Hegazy, M. A. A. Mortada, M. R. Hegazy, and M. Helal. 1978. The use of *Aloe vera* extract in the treatment of experimental corneal ulcers in rabbit. *J. Drug Res. Egypt* 10:199–209.

Heggers, J. P., G. R. Pineless, and M. C. Robson. 1979. Dermaide Aloe/*Aloe vera* Gel: Comparison of the antimicrobial effects. *J. Am. Med. Technol.* 41:293.

Heidemaan, A., H. G. Miltenburger, and U. Mengs. 1993. The genotoxicity of Senna. *Pharmacology* 47:179–186, Suppl 1.

Heidemaan A., W. Voelkner, and U. Mengs. 1996. Genotoxicity of aloe emodin in vitro and in vivo. *Mutat. Res.* 367:123–133.

Herlihy, J. T., H. A. Bertrand, J. D. Kim, J. Ikeno, and B. P. Yu. 1998. Effects of *Aloe vera* ingestion in the rat. I. Growth, food and fluid intake and serum chemistry. *Phytother. Res.* 12:183–188.

Hikino, H., M. Takahashi, M. Murakami, C. Konno, et al. 1986. Isolation and hypoglycemic activity of arborans A and B, glycans of *Aloe arborescens* var Natalensis leaves. *Int. J. Crude Drug Res.* 24:183–186.

Hirata, T., S. Sakano, and T. Suga. 1981. Biotransformation of aloenin, a bitter glucoside constituent of *Aloe arborescens*, by rats. *Experientia* 37:1252–1253.

Hirata, T., and T. Suga. 1977. Biologically active constituents of leaves and roots of *Aloe arborescens* var natalensis. *Z Naturforsch.* 32:731–734.

Hogan, D. J. 1988. Widespread dermatitis after topical treatment of chronic leg ulcers and stasis dermatitis. *Can. Med. Assoc. J.* 138:336–338.

Holdsworth, D. K. 1971. Chromones in Aloe species. *Planta Med.* 19:322–325.

Hunter, D., and A. Frumkin. 1991. Adverse reactions to vitamin E and *Aloe vera* preparations after dermabrasion and chemical peel. *Cutis* 47:193–196.

Hutter, J. A., M. Salman, W. B. Stavinoha, et al. 1996. Anti-inflammatory C-glucosyl chromone from *Aloe barbadensis*. *J. Nat. Prod.* 59:541–543.

Imanishsi, K., T. Ishiguro, H. Saito, and I. Suzuki. 1981. Pharmacological studies on a plant lectin, Aloctin A. I. Growth and inhibition of mouse methylcholanthrene-induced fibrosarcoma (Meth A) in ascites form by Aloctin A. *Experientia* 37:1186–1187.

Institut D'Expertise Clinique. 2000. Ocular tolerant assessment and evaluation of the primary cutaneous tolerance. Unpublished data. 12 pages.[2]

Institut Francais de Recherches et Essais Biologoques. 1981.Ocular irritation in the rabbit and cutaneous primary irritation in the rabbit. Unpublished data. 32 pages.[2]

International Aloe Science Council (IASC). 2001a. Polymerase chain reaction: A new method for the identification of aloe. http://www.iasc.org/orndorf.html.

IASC. 2001b. *Aloe vera*, Quality inspection and identification. www.iasc.org.

IASC. 2003. IASC addresses aloe nomenclature for CTFA. *Inside Aloe* September, 2003. www.iasc.org.

Ishii, Y., H. Tanizawa, and Y. Takino. 1984. Fluorophotometry of barbaloin in aloe. *Chem. Pharm. Bull. (Tokyo).* 32:4946–4950.

Ishii, Y., H. Tanizawa, and Y. Takino. 1987. Determination of barbaloin in rat serum. *Chem. Pharm. Bull. (Tokyo).* 35:4642–4644.

Ishii, Y., H. Tanizawa, and Y. Takino. 1990. Studies of *Aloe*. III. Mechanism of laxative effect. *Chem. Pharm. Bull*. 38:197–200.

Ishii, Y., H. Tanizawa, and Y. Takino. 1994. Studies of *Aloe*. V. Mechanism of cathartic effect. *Biol. Pharm. Bull*. 17:651–653.

Ivy Laboratories. 1987a. The determination of the photocontact allergenic potential of 0.1% Aloe extract in product type 77. Unpublished data. 13 pages.[2]

Ivy Laboratories. 1987b. The determination of the phototoxic potential of 0.1% Aloe extract in product type 77. Unpublished data. 15 pages.[2]

Ivy Laboratories. 1988. The determination of the photocontact allergenic potential of 0.01% Aloe Barbadensis flower extract in product type 77. Unpublished data. 15 pages.[2]

Ivy Laboratories. 1996. The determination of the photocontact allergenic potential of 0.5% Aloe extract in product type 79. Unpublished data. 12 pages.[2]

Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry on Recommended Methods for the Evaluation of Drugs. 1967. The chemical assay of Aloes. *Analyst* 92:593–596.

Karaca, K., J. M. Sharma, and R. Norgen. 1995. Nitric oxide production by chicken macrophages activated by acemannan, a complex carbohydrate extracted from *Aloe vera*. *Int. J. Immunopharmacol*. 17:183–188.

KGL, Inc. 2000. The determination of the photocontact allergenic potential of 0.1% Aloe extract in product type 77. Unpublished data. 16 pages.[2]

KGL, Inc. 2001. The determination of the photocontact allergenic potential of 0.5% Aloe extract in product type 79. Unpublished data. 18 pages.[2]

Kacew, S. 1993. Neonatal toxicology. *Gen. Appl. Toxicol*. 2:1047–1068.

Kahlon, J. B., M. C. Kemp, R. H. Carpenter, et al. 1991a. Inhibition of AIDS virus replication by acemannan in vitro. *Mol. Biother.* 3:127–135.

Kahlon, J. B., M. C. Kemp, and N. Yawei. 1991b. In vitro evaluation of the synergistic antiviral effects of acemannan in combination with azidothymidine and acyclovir. *Mol. Biother.* 3:211–220.

Kar, A., S. Panda, and S. Bharti. 2002. Relative efficacy of three medicinal plant extracts in the alteration of thyroid hormone concentrations in male mice. *J. Ethnopharmacol*. 81:281:285.

Kim, H. S., S. Kacew, and B. M. Lee. 1999. In vitro chemopreventive effects of plant polysaccharides (*Aloe barbadensis* Miller, Lentinum edodes, Ganoderma lucidum and Coriolus versicolor). *Carcinogenesis* 20:1637–1640.

Kim, H. S., and B. M. Lee. 1997. Inhibition of benzo [a] pyrene-DNA adduct formation by *Aloe barbadensis* Miller. *Carcinogenesis* 18:771–776.

Kim, K. H., J. G. Lee, D. G. Kim, et al. 1998. The development of a new method to detect the adulteration of commercial aloe gel powders. *Arch. Pharm. Res*. 21:514–520.

King, G. K., K. M. Yates, P. G. Greenlee, et al. 1995. The effect of acemannan immunostimulant in combination with surgery and radiation therapy on spontaneous canine and feline fibrosarcomas. *J. Am. Anim. Hosp. Assoc*. 31:439–447

Klein, A. D., and N. S. Penneys. 1988. *Aloe vera*. *J. Am. Acad. Dermatol*. 18:714–20.

Koo, M. W. 1994. *Aloe vera*: Anti-ulcer and anti-diabetic effects. *Phytother. Res*. 8:461–464.

Kuo, P.-L., T.-C. Lin, and C.-C. Lin. 2002. The antiproliferative activity of aloe-emodin is through p53-dependent and p21-dependent apoptotic pathway in human hepatoma cell lines. *Life Sci*. 16:1879–1892.

Kuzuya, H., I. Tamai, H. Beppu, et al. 2001. Determination of aloenin, barbaloin, and isobarbaloin by micellar electrokinetic chromatorgraphy. *J. Chromatogr. B Biomed. Sci. Appl*. 752:91–97.

Lagarto Parra, A., R. Yhebra, G. Sardinas, et al. 2001. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50}) in mice to determine oral acute toxicity of plant extracts. *Phytomedicine* 8:395–400.

Lang, W. 1993. Pharmacokinetic-metabolic studies with ^{14}C-aloe-emodin after oral administration to male and female rats. *Pharmacology.* 47:110–119, suppl 1.

Lee, C. K., S. S. Han, Y. K. Mo, et al. 1997. Prevention of ultraviolet radiation-induced suppression of accessory cell function of Langerhans cells by *Aloe vera* gel components. *Immunopharmacology* 37:153–162.

Lee, C. K., S. S. Han, Y. K. Shin, et al. 1999. Prevention of ultraviolet radiation-induced suppression of contact hypersensitivity by *Aloe vera* gel components. *Int. J. Immunopharmacol*. 21:303–310.

Lee, H.-Z., 2001. Protein kinase C involvement in aloe-emodin and emodin-induced apoptosis in lung cell carcinoma cell. *Br. J. Pharmacol*. 5:1093–1103.

Lee, H.-Z., S.-L. Hsu, M.-C. Liu, and C.-H. Wu. 2001. Effects and mechanisms of aloe-emodin on cell death in human lung squamous cell carcinoma. *Eur. J. Pharmacol*. 23:287–295.

Lee, J. K., M. K. Lee, Y.-P. Yun, et al. 2001. Acemannan purified from *Aloe vera* induces phenotypic and functional maturation of immature dendritic cells. *Int. Immunopharmacol*. 1:1275–1284

Lee, K. Y., J. H. Park, M. H. Chung, et al. 1997. Aloesin up-regulates cyclin E/CDK2 kinase activity via inducing the protein levels of Cyclin E, CDK2 and CDC25A in SK-HEP-1cells. *Biochem. Mol. Biol. Int*. 41:285–292.

Lee, M. J., S. H. Yoon, S. K. Lee, et al. 1995. In vivo angiogenic activity of dichloromethane extracts of *Aloe vera* gel. *Arch. Pharm. Res*. 18:332–335.

Lissoni, P., L. Giani, S. Zerbini, P. Trabattoni, and F. Rovelli. 1998. Biotherapy with the pineal immunomodulating hormone melatonin versus melatonin plus *Aloe vera* in untreatable advanced solid neoplasms. *Nat. Immunol*. 16:27–33.

Lorenzett, L. J., R. Salisbury, J. Beal, and J. Baldwin. 1964. Bacteriostatic property of *Aloe vera*. *J. Pharm. Sci*. 53:1287.

Loveman, A. B. 1937. Leaf of *Aloe vera* in treatment of roentgen ray ulcers. *Arch. Dermatol. Syphilol*. 36:838–843.

Mabusela, W. T., A. M. Stephen, and M. C. Botha. 1990. Carbohydrate polymers from *Aloe ferox* leaves. *Phytochemistry* 29:3555–3558.

Madis, V. H., and M. M. Omar. 1989. Aleoferon isolation, manufacturing and its application. United States Patent 4,861,761.

Makino, K., A. Yagi, and I. Nishioka. 1973. Studies on the constituents of *Aloe arborescens* (Miller): The structure of aloearbonaside, a glucoside of a new type naturally occurring chromene. *Chem. Pharm. Bull*. 21:149–156.

Mandeville, F. B. 1939. *Aloe vera* in the treatment of radiation ulcers of mucous membranes. *Radiology* 32:598–599.

Marshal, G. D., A. S. Gibbons, and L. S. Parnell. 1993. Human cytokines induced by acemannan (abstract 619). *J. Allergy Clin. Immunol*. 91:295.

Maze, G., R. N. Terpolilli, and M. Lee. 1997. *Aloe vera* extract prevents aspirin-induced acute gastric mucosal injury in rats. *Med. Sci. Res*. 25:765–766.

McAnalley, B. H. 1990. Processes for preparation of Aloe products, products produced thereby and composition thereof. United States Patent 4,917,890.

McCarthy, T. J. 1969. Distribution of glycosyl compounds in South African aloe species. *Planta Med.* 17:1–7.

McCarthy, T. J., and L. J. Haynes. 1967. The distribution of aloesin in some South African aloe species. *Planta Med*. 15:342–344.

McCauley, R., J. P. Haggers, and M. C. Robson. 1990. Frostbite-methods to minimize tissue loss. *Postgrad. Med*. 88:67–70.

MDS Pharma Services. 2000. Single dose toxicity study by the oral route in the rat (Limit test). Unpublished data. 30 pages.[2]

Meadows, T. P. 1980. Aloe as a humectant in new skin preparations. *Cosmet. Toilet*. 95:51–56

Ministry of Health, Labor and Welfare (MHLW). March 2001a. Unofficial translation of MHW Ordinance No. 331, Attached Table 1 [Negative List]. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2, 1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100–8045. Japan.

MHLW. March 2001b. Unofficial translation of MHW Ordinance No. 331, Attached Table 2 [Restricted List]. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2, 1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100–8045. Japan.

Morimoto, I., F. Wantanabe, and T. Osawa. 1982. Mutagenicity screening of crude drugs with *Bacillus subtilis* rec-assay and *Salmonella*/microsome reversion assay. *Mutat. Res.* 97:81–102.

Morrow, D. M., M. J. Rapaport, and R. A. Strick. 1980. Hypersensitivity to Aloe. *Arch. Dermatol.* 116:1064–1065.

Müller, S. O., I. Eckert, W. K. Lutz, and H. Stopper. 1996. Genotoxicity of the laxative drug components emodin, aloe-emodin and danthron in mammalian cells: Topoisomerase II mediated? *Mutat. Res.* 371:165–173.

Nakamura, T., and S. Kotajima. 1984. Contact dermatitis from *Aloe arborescens*. *Contact Dermatitis* 11:51.

Nakamura, H., and T. Okuyama. 1990. Gas chromatographic and mass spectral determination of aloenin in skin-care cosmetics. *Chromatography* 509:377–382.

Nakano, M., T. Yamagishi, T. Takahashi, and H. Kaneshima. 1985. Analysis of plant components in cosmetics. Part I. Determination of aloenin, a component of *Aloe arborescens* Mill in Lotion. *Hokkaidoritsu Eisei Kenkyushoh.* 35:98–101.

Nath, K., N. Sethi, R. K. Singh, and A. K. Jain. 1992. Commonly used Indian abortifacient plants with special reference to their teratologic effects in rats. *J. Ethnopharmacol.* 36:147–154.

Natow, A. J. 1986. *Aloe vera*, fiction or fact. *Cutis* 37:106, 108.

Norikuri, T., D. O. Kennedy, A. K. Nyarko, A. Kojima, and I. Matsui-Yuasa. 2002. Protective effect of aloe extract against the cytotoxicity of 1,4-napthoquinone in isolated rat hepatocytes involves modulation in cellular thiol levels. *Pharmacol. Toxicol.* 5: 278–284.

Northway, R. B. 1975. Experimental use of *Aloe vera* in clinical practice. *Vet. Med. Small Anim. Clin.* 70:89.

Norton, S. J., V. Talesa, and W. J. Yuan. 1990. Glyoxalase I and glyoxalase II from *Aloe vera*: Purification, characterization and comparison with animals Glyoxalases. *Biochem. Int.* 22:411-418.

Olsen, D. L., W. Raub Jr., C. Bradley, et al. 2001. The effect of *Aloe vera* gel/mild soap versus mild soap alone in preventing skin reactions in patients undergoing radiation therapy. *Oncol. Nurs. Forum.* 28:543–547.

Parmar, N. S., M. Tariq, M. A. Al-Yahya, et al. 1986. Evaluation of *Aloe vera* leaf exudates and gel for gastric and duodenal anti-ulcer activity. *Fitoterapia* 57:380–383.

Parry, O., and C. Matambo. 1992. Some pharmacological actions of aloe extracts and Cassia abbreviata on rats and mice. *Cent. Afr. J. Med.* 38:409–414.

Patri, F., and V. Silano. 2002. *Plants in Cosmetics, plants and plant preparations used as ingredients for cosmetic product,* 79–80. Strasbourg: Council of Europe Publishing.

Pecere, T., M. V. Gazzola, C. Mucignat, et al. 2000. Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. *Cancer Res.* 60:2800–2804.

Peny, S. Y., J. Normal, G. Curtin, et al. 1991. Decreased mortality of Norman murine sarcoma in mice treated with the immunomodulator acemannan. *Mol. Biother.* 3:79–87.

Proserpi, G. 1976. Natural sunscreens: vegetable derivatives as sunscreens and tanning agents. *Cosmet. Toilet.* 91:34–36.

Pugh, N., S. A. Ross, M. A. ElSohly, and D. S. Pasco. 2001. Characterization of aloeride, a new high molecular weight polysaccharide from *Aloe vera* with potent immunostimulatory activity. *J. Agric. Food Chem.* 49:1030–1034.

Qiu, Z., K. Jones, M. Wylie, et al. 2000. Modified *Aloe barbadensis* polysaccharide with immunomodulatory Activity. *Planta Med.* 66:152–156.

Ramamoorthy, L., and I. R. Tizard. 1998. Induction of apoptosis in a macrophage cell line RAW 264.7 by acemannan, a β (1,4) acetylated mannan. *Mol. Pharmacol.* 3:415–421.

Reynolds, T., and A. C. Dweck. 1999. *Aloe vera* leaf Gel: A review update. *J. Ethnopharmacol.* 68:3–37.

Roberts, D. B., and E. L. Travis. 1995. Acemannan-containing wounddressing gels reduce radiation-induced skin reactions in C3H mice. *Int. J. Radiat. Oncol. Biol. Phys.*15:1047–1052.

Roboz, E., and A. J. Haagen-Smit. 1948. A mucilage from *Aloe vera*. *J. Am. Chem. Soc.* 70:3248–3249.

Robson, M. C., J. Heggers, and W. J. Hagstrom. 1982. Myth, magic, witchcraft or fact? *Aloe vera* revisited. *J. Burn Care Rehab.* 3:157–162.

Ross, S. A., M. A. ElSohly, and S. P. Wilkins. 1997. Quantitative analysis of *Aloe vera* mucilagenous polysaccharides in commercial *Aloe vera* products. *J. AOAC Int.* 80:455–457.

Rowe, T. D., and L. M. Parks. 1941. Phytochemical study of *Aloe vera* leaf. *J. Am. Pharm. Assoc.* 30:262–266.

Sabeh, F., T. Wright, and S. J. Norton. 1993. Isoenzymes of superoxide dismutase from *Aloe vera*. *Enzyme Protein* 47:92–98.

Sabeh, F., T. Wright, and S. J. Norton. 1996. Purification and characterization of a glutathione peroxidase from *Aloe vera* plant. *Enzyme Protein* 49:212–221.

Saleem, R., S. Faizi, B. S. Siddiqui, et al. 2001. Hypotensive effects of chemical constituents from *Aloe barbadensis*. *Planta Med.* 67:757–760.

Saito, H., K. Imanishi, and S. Okabe. 1989. Effects of aloe extracts, aloctin A, on gastric secretion and on experimental gastric lesions in rats. *Yakugaku Zasshi* 109:335–339.

Sauchak, U. I. 1977. Acute bulbous allergic dermatitis due to local application of aloe leaves. *Vestn. Dermatol. Venerol.* 12:44–45.

Shah, A. H., S. Qureshi, M. Tariq, and A. M. Ageel. 1989. Toxicity studies on six plants used in the traditional Arab system of medicine. *Phytother. Res.* 3:25–29.

Shamaan, N. A., K. A. Kadir, A. Rahmat, and W. Z. Ngah. 1998. Vitamin C and *Aloe vera* supplementation protects from chemical hepatocarcinogenesis in the rat. *Nutrition* 14:846–852.

Sheets, M. A., B. A. Unger, G. F. J. Giggleman, and I. R. Tizard. 1991. Studies on the effect of acemannan on retrovirus. *Mol. Biother.* 3:41–45.

Shelton, R. M. 1991. *Aloe vera* its chemical and therapeutic properties. *Int. J. Dermatol.* 30:679–683.

Shida, T., A. Yagi, H. Nishimura, and I. Nishioka. 1985. Effect of *Aloe vera* Extract on peripheral phagocytosis in adult bronchial asthma. *Planta Med.* 36:273–275.

Shimpo, K., T. Chihara, H. Beppu, C. Ida, T. Haneko, T. Nagatsu, and H. Kuzya. 2001. Inhibition of azoxymethane-induced aberrant crypt foci formation in rat colorectum by whole leaf *Aloe arborescens* Miller var. *natalensis* Berger. *Phytother. Res.* 15:705–711.

Shimpo, K., C. Ida, T. Chihara, H. Beppu, T. Kaneko, and H. Kuzuya. 2002. *Aloe arborescens* extract inhibits TPA-induced ear oedema, putrescine increase and tumor promotion in mouse skin. Short communication. *Phytother. Res.* 16:491–493.

Shiseido Safety Research Labs. 2002. Photoallergenicity of Aloe Arborescens CRS. Unpublished data submitted by CTFA. 9 pages.[2]

Shoji, A. 1982. Contact Dermatitis to *Aloe arborescens*. *Contact Dermatitis* 8:164–167.

Siegers, C. P., J. Siemers, and G. Baretton. 1993. Sennosides and aloin do not promote dimethylhydrazine induced colorectal tumors in mice. *Pharmacology* 47:205–208, Suppl 1.

Singh, R. P., S. Dhanalakshmi, and A. R. Rao. 2000. Chemomodulatory action of *Aloe vera* on the profiles of enzymes associated with carcinogen metabolism and antioxidant status regulation in Mice. *Phytomedicine* 7:209–219.

Strickland, F. M., A. Darvill, P. Albersheim, et al. 1999. Inhibition of UV-induced immune suppression and interleukin-10 production by plant oligosaccharides and polysaccharides. *Photochem. Photobiol.* 69:141–147.

Strickland, F. M., H. K. Muller, L. C. Stephens, et al. 2000. Induction of primary cutaneous melanomas in C3H mice by combined treatment with ultraviolet radiation, ethanol and aloe-emodin. *Photochem. Photobiol.* 72:407–414.

Strickland, F. M., R. P. Pelley, and M. L. Kripke. 1994. Prevention of ultraviolet radiation induced suppression of contact and delayed hypersensitivity by *Aloe barbadensis* gel extracts. *J. Invest. Dermatol.* 102:197–204.

Sturm, P. G., and S. M. Hayes. 1984. *Aloe vera* In dentistry. *J. Bergen County Dent. Soc.* 50:11–14.

Suga, T., and T. Hirata. 1983. The efficacy of the Aloe plant chemical constituents and biological activities. *Cosmet. Toilet.* 98:105–108.

Suga, T., T. Hirata, and M. Odan. 1972. Aloenin, a new bitter glucoside from aloe species. *Chem. Lett.* 547.

Suga, T., T. Hirata, and K. Tori. 1974. Structure of aloenin, a bitter glucoside from aloe species. *Chem. Lett.* 715.

Suzuki, I., H. Saito, S. Inoue, S. Migita, and T. Takahashi. 1979. Purification and characterization of two lectins from *Aloe arborescens* Mill. *J. Biochem. (Tokyo)* 85:163–171.

Sydiskis, R. J., D. G. Owen, J. L. Lohr, et al. 1991. Inactivation of enveloped viruses by anthraquinones extracted from plants. *Antimicrob. Agents Chemother.* 35:2463–2466.

Syed, T. A., M. Afzal, S. A. Ahmad, et al. 1997. *Aloe vera* extract 0.5% in a hydrophilic cream versus *Aloe vera* gel for the management of genital herpes in males. A placebo controlled double blind comparative study. *J. Dermatol. Treat.* 8:99–102.

Syed, T. A., S. A. Shamd, A. H. Holt, et al. 1996. Management of psoriasis with *Aloe vera* extract in a hydrophilic cream: a placebo-controlled, double blind study. *Trop. Med. Int. Health.* 1:505–509.

Tyler, V. E. 1994. *Herbs of choice*, 155–157. New York: Pharmaceutical Products Press.

Tyler, V. E., L. R. Bradley, and J. E. Robbers, ed. 1988. *Pharmacognosy*, 9th ed., 62–63. Philadelphia: Lea & Febiger.

Tello, C. G., P. Ford, and A. M. Iacopino. 1998. In vitro evaluation of complex carbohydrate denture adhesive formulations. *Quintessence Int.* 29:585–593.

Teradaira, R., M. Shinzato, H. Beppu, and K. Fujita. 1993. Anti-gastric ulcer effect of *Aloe arborescens* Mill. Var natalensis Berber. *Phytother. Res.* 7:S34–S36.

t'Hart, L. A., P. H. van Enckevort, H. van Dijk, and R. P. Labadie. 1988. Two functionally and chemically distinct immunomodulatory compounds in the gel of *Aloe vera*. *J. Ethnopharmacol.* 23:61–71.

Thomas, D. R., P. S. Goode, K. LaMaster, and T. Tennyson. 1998. Acemannan hydrogel dressing versus saline dressing for pressure ulcers. *Adv. Wound Care.* 11:273–276.

Tizard, A. U., D. Busbee, B. Maxwell, and M. C. Kemp. 1995. Effect of acemannan, a complex carbohydrate, on wound healing in young and aged rats. *Wounds* 6:201–209.

Udupa, S. I., A. L. Udupa, and D. R. Kulkarni. 1994. Anti-inflammatory and wound healing properties of *Aloe vera*. *Fitoterapia* 65:141–145.

Uehara, N., Y. Iwahori, M. Asamoto, et al. 1996. Decreased levels of 2-amino-3methylimidazo[4,5-f]quinoline-DNA adducts in rats treated with β-carotene, α-tocopherol and rreeze dried Aloe. *Jpn. J. Cancer Res.* 87:342–348.

UNITIS. 2003. Unpublished data submitted by European Organizatioon of Cosmetic Ingredients Industries and Services, Paris. 31 pages.[2]

USDA, ARS, National Genetic Resource Program. Germplasm Resources Information Network (GRIN). [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. Internet site accessed August, 2003 http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl

van Wyk, B. E., M. C. B. Oudtshoom, and G. F. Smith. 1995. Geographical variation in the major compounds of *Aloe ferox* exudate. *Planta Med.* 61:250–253.

Vargas F., G. Fraile, M. Velasquez, et al. 2002. Studies on the photostability and phototoxicity of aloe-emodin, emodin and rhein. *Pharmazie* 57:399–404.

Vath, P., W. G. Wamer, and D. E. Falvey. 2002. Photochemistry and photoxicity of aloe-emodin. *Photochem. Photobiol.* 75:346–352.

Viljoen, A. M., B. V. Van Wyk, and L. E. Newton. 2001. The occurrence and taxonomic distribution of the anthrones aloin, aloinoside and microdotin in aloe. *Biochem. Syst. Ecol.* 29:53–67.

Visuthikosol, V., B. Chowchuen, Y. Sukwanarat, et al. 1995. Effect of *Aloe vera* gel to healing of burn wound in a clinical and histologic study. *J. Med. Assoc. Thai* 78:403–409.

Vogler, B. K., and E. Ernst. 1999. *Aloe vera*: a systematic review of its clinical effectivness. *Br. J. Gen. Pract.* 49:823–828.

Waller, G. R., S. Mangiafico, and C. R. Ritchey. 1978. A chemical investigation of *Aloe barbadensis* miller. *Proc. Okla. Acad. Sci.* 58:69–76.

Wasserman, L., S. Avigad, E. Beery, J. Nordenberg, and E. Fenig. 2002. The effect of aloe emodin on the proliferation of a new Merkel cell carcinoma cell line. *Am. J. Dermopathol.* 24:17–22.

Westendorf, J., H. Marquardt, B. Paginsky, et al. 1990. Genotoxicity of naturally occuring hydroxyahthraquinones. *Mutat. Res.* 240:1–12.

Williams, M. S., M. Burk, C. L. Loprinzi, et al. 1996. Phase III double blind evaluation of an *Aloe vera* gel as a prophylactic agent for radiation-induced skin toxicity. *Int. J. Radiat. Oncol. Biol. Phys.* 36:345–349.

Winters, W. D., R. Benavides, and W. J. Clouse. 1981. Effects of aloe extracts on human normal and tumor cells in vitro. *Econ. Bot.* 35:89–95.

Woo, S. W., J. X. Nan, S. H. Lee, E. J. Park, Y. Z. Zhao, and D. H. Sohn. 2002. Aloe emodin supresses myofibroblastic differentiation of rat hepatic stellate cells in primary culture. *Pharmacol. Toxicol.* 90:193–198.

Wright, C. S. 1936. Leaf of *Aloe vera* in treatment of roentgen ulcers and telangiectasis. *J. Am. Med. Assoc.* 103:1363–1364.

Yagi, A., T. Egusa, M. Arase, M. Tanabe, and H. Tsugi. 1997. Isolation and characterization of the glycoprotein fraction with a proliferation-promoting activity on human and hamster cells in vitro from *Aloe vera* gel. *Planta Med.* 63:18–21.

Yagi, A., N. Harada, K. Shimomura, and I. Nishioka. 1987. Bradykinin-degrading glycoprotein in *Aloe arborescens* var. *natalensis*. *Planta Med.* 53:19–21.

Yagi, A., K. Machii, H. Nishimura, et al. 1985. Effect of aloe lectin on deoxyribonucleic acid synthesis in baby hamster kidney cells. *Experientia* 41:669–671.

Yagi, A., K. Makino, I. Nishioka, and Y. Kuchino. 1977. Aloe mannan, polysaccharide, from *Aloe arborescens* var. *natalensis*. *Planta Med.* 31:17–20.

Yagi, A., J. Nakamori, T. Yamada, et al. 1999. In vivo metabolism of Aloemannan. *Planta Med.* 65:417–420.

Yagi, A., K. Sato, K. Akasaki, and H. Tsuji. 2000. Distribution of verectin in *Aloe vera* leaves and verectin contents in clonally regenerated plants and the commercial gel powders by immunochemical screening. *Planta Med.* 66:180–182.

Zawahry, M. E., M. R. Hegazy, and M. Helal. 1973. Use of aloe in treating leg ulcers and dermatoses. *Int. J. Dermatol.* 12:68–73.

Zhang, L., and I. R. Tizard. 1996. Activation of a mouse macrophage cell line by acemannan: The major carbohydrate fraction from *Aloe vera* gel. *Immunopharmacology* 35:119–128.