Identification of Some Prostanoids in *Aloe vera* Extracts

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Abstract

Screening of extracts from *Aloe vera* revealed the presence of endogenous arachidonic acid, a potential precursor for the prostanoids synthesis. The presence of endogenous cyclooxygenase, in the plant extracts, was established by radiometric assay. Relatively high proportions of PGE_2 and TXB_2 and low proportions of other prostaglandins were identified in the plant extract when incubated with [^{14}C]-arachidonic acid. Other lipids present in this plant were examined and a high percentage of phosphatidylcholine and cholesterol was established. Possible importance of prostaglandins formed endogenously by the plant is discussed.

Key words

Aloe vera, arachindonic acid, prostanoid synthesis.

Introduction

Several plants are used in the Middle Eastern and South East Asian countries for the treatment of boils and other skin affections. Reports of the healing virtues of *Aloe vera* L. (Liliaceae) date back to the writings of Hippocrates and Alexander the Great (1). One recent study shows that the chemicals in *Aloe vera* extract produce an anesthetic reaction, kill bacteria and increase local microcirculation (2). Plants belonging to *Aloes* have been used in many countries for the folklore treatment of inflamed eyes (3) and wound healing (4, 5) by topical application of the fresh gel from the plant, as a pugative due to its anthraquinone glycosides barbaloin, beta-barbaloin and homonatloin (6, 7), treatment of chronic skin ulcers, stasis dermatitis (8), acne-induced osteoma cutis, and the main ingredient in tincture benzoin (9). Antiarthritic activity of anthraquinones in *Aloe* for podiatric medicine, and osteoarthrosis has been reported (10).

Hypoglycemic activity of *Aloes* has also been recently demonstrated (11, 12). In addition the prophylactic and therapeutic effect of *Aloe* extract on leukopenia caused by exposure to cobalt 60 and its antitumor activity against sarcoma-180 and Ehrich ascites have been reported (13). The report on peripheral phygocytosis in adult bronchial asthma and persistent cough in pharyngitis (14), attracted our attention to relook into this potentially important activity.

There is evidence to show that during the storage period of the plant extract some potent compound is formed that is responsible for its efficacy in chronic bronchial asthmatic patients as well as intrinsic types of various ages (14). Although Shida and coworkers (14) have suggested that this potent component could be a high molecular mass compound partially identified as a glycoprotein and/or a neutral polysaccharide (15), Yagi and coworkers (16) have reported a bradykinin-degrading glycoprotein in *Aloe*. This was particularly important in a situation when the extract was potent only for those patients who were not pretreated with steroid drugs. These and other observations led us to investigate the presence of prostaglandins in *Aloe vera* extracts. In this endeavor, we are reporting on the lipid components and the presence of cyclooxygenase in *Aloe* extracts.

Materials and Methods

Aloe vera was cultivated under greenhouse conditions at Kuwait University. Identity of the plant was established by Iftakhar Ahmad Niazi, Department of Botany and Microbiology, University of Kuwait. All solvents were of analytical grade and except acetic acid, were redistilled before use. Lipid fatty acid methyl esters and prostaglandin standards were purchased from Sigma Chemicals U.S.A. [^{14}C]-arachidonic acid was an Amersham product. Thin layer chromatograms used in this study were aluminium coated plates without fluorescent indicator and were a product of E. Merck. A Desaga TLC scanner linked with a Spectra Physics integrator was used for scanning the TLC chromatograms. The TLC radiochromatograms were scanned with a Packard scanner. A Perkin-Elmer gas liquid chromatograph (GLC), linked with a Sigma 3 data station, was used for the fatty acid methyl ester analyses. A 50 meters capillary column coated with 10% DEGS was used for this study. The column temperature was maintained at 180 °C while the detector temperature was 250 °C. Helium gas flow rate of 2 ml was maintained throughout this study.

Lipid extraction

Small pieces of the overground part of the fresh plant (500 g), rich in yellow exudate, were homogenised by polytron homogenizer and extracted by the Folch method (17). The extract was filtered and the residue thoroughly washed with fresh cold solvent. The extract was washed with a normal saline solution. The solvent was evaporated under reduced pressure at 30 °C to give

the total lipid extract. The nonlipid material was removed by percolating the extract solution through a Sephadex G-25 column. This, after removal of the final traces of the solvent under nitrogen, yielded the total lipid content of the plant. The crude mixture was fractionated into nonpolar and polar fractions on a silica gel column by elution with petroleum spirit : chloroform (9 : 1, v/v) followed by methanol, respectively.

The nonpolar lipids were resolved on TLC in petroleum spirit : diethyl ether : acetic acid (9 : 1 : 1, v/v). The chromatograms were visualized by a mixture of 30% methanolic sulfuric acid followed by heating the chromatograms at 120 °C. The glycolipids were resolved in the two dimensional solvent system, chloroform : methanol : water (65 : 25 : 4, v/v) and chloroform : methanol : acetone : acetic acid (60 : 10 : 20 : 5, v/v). The chromatograms were sprayed with anthrone or phosphomolybdic acid for scanning. Phospholipids were resolved in a solvent system containing chloroform : methanol : acetic acid : water (170 : 25 : 25 : 6, v/v). Phospholipids were also resolved into its components by a two dimensional TLC in the same solvent system as described for the glycolipids.

Methyl esters of the fatty acids were prepared by transesterification using methanolic hydrochloric acid and benzene (3 : 2, v/v). The different FAMEs were identified from their relative retention times compared with standards, run under identical conditions as well as from the separation factors of the unknown with those of the known FAMEs.

Studies on cyclooxygenase

The fresh plant (10 g) was slashed into small pieces and homogenized in cold Tris buffer (0.05 M, pH 7.0). The extract was centrifuged at 3000 rpm for 10 min and the supernatant collected. Tubes containing 5 ml of the extract were preincubated for five minutes with or without indomethacin. Synthesis of prostaglandins was initiated by the addition of [^{14}C]-arachidonic acid mixed with unlabelled arachidonic acid. The incubation was carried out at 37 °C for 30 min. The reaction was stopped and its pH adjusted to 3.0 with 9.2% formic acid. The total lipid mixture was extracted three time with 3 ml of ethyl acetate. The extracted samples were dried under nitrogen and the labelled products were separated from the labelled precursor using mini silicic acid columns as described earlier (18). The fractions containing prostaglandins and thromboxanes were resolved on TLC chromatograms in solvent system containing ethyl acetate : isopentane : acetic acid : water (110 : 50 : 20 : 100, v/v) versus standard prostaglandins. The TLC chromatograms were scanned on a Packard model 7201 radiochromatogram scanner. The corresponding spots were eluted and counted in a liquid scintillation counter.

Results and Discussion

Column chromatographic separation of the total lipids (35.5 g) of the plant extract (500 g) yielded 3.5 g of nonpolar lipids. The composition of this fraction was established by scanning the thin layer chromatograms in solvent system: petroleum spirit : diethyl ether : acetic acid (9 : 1 : 0.1, v/v) and is given in Table 1.

Table 1 Composition of the nonpolar lipids of *Aloe vera*.

Cholesterol	12.50%
Stigma sterol	18.40%
Stigma stearate	21.30%
Methyl oleate	7.10%
Triolein	2.00%
Oleic acid	1.30%

The presence of cholesterol in *Aloe vera* is rather interesting since not very many plants are known to contain cholesterol as their lipid component. Plant sterols are known to fit into the phospholipid leaflet which comprises the monolayer component of the bilayer arrangement of natural membranes. While animals have cholesterol as their major lipid, in flowering poinsettia plants half of the sterol is identified as cholesterol, in croton seed oil 93% of the sterol is ethylcholesterol (19) and in vine *Clerodendrum splendens*, nearly all of the sterol has been identified as 25(27)-dehydroporiferasterol (20).

Phospholipid fractionation from the column afforded 4.6 g of yellowish material. This mixture consisted of eight components, as shown by TLC in various solvent systems. The different components of this mixture were identified by running the standards along with the mixture on the same chromatograms. Identification and scanning of the chromatograms revealed the presence of the polar lipids as shown in Table 2.

Table 2 Composition of polar lipids of *Aloe vera*.

Phosphatidyl choline (PC)	12.05%
Phosphatidyl ethanolamine (PE)	12.03%
Phosphatidic acid (PA)	47.30%
Phosphatidyl serine (PS)	6.50%
Phosphatidyl inositol (PI)	2.70%
Lysophosphatidyl inositol (LPI)	1.20%
Sphingomyelin (SP)	4.20%
Sulfoquinovosyl diglyceride (SQD)	16.80%

It is a common observation that PE is the major phospholipid in most living organisms including plants. In fish PC and PE constitute 32% of the total lipids (21). In our analysis of *Aloe vera* a high percentage of PC equal to that of PE makes this plant rather unusual probably suggesting an adaptation of the plant to the desert climate. It is known that the ability of seedlings to resist extreme temperatures can be enhanced by exposure to amino alcohols such as choline or ethanolamine. It has been shown to increase the plant content of PC at the expense of PE (22). It has also been reported that PC mostly contains saturated fatty acids such as palmitate and stearate while unsaturated fatty acids, such as arachidonate and dodecosahexaenoate are the components of PE (23).

A high percentage of phosphatidic acid in *Aloe vera* is also unique and is probably derived from the major phospholipids after their decomposition. While the presence of sulfoquinovosyl diglyceride in comparatively high yield, again suggests the adaptation of this plant to the desert conditions since this lipid has been implicated as the savior of plants under dry conditions.

Table 3 Fatty acid composition of *Aloe vera* lipids.

16:0	4.2%	16:1	4.9%	16:2	2.8%
18:0	3.4%	18:1	9.3%	18:2	15.6%
18:3	41.7%	20:0	1.2%	20:4	3.1%
24:0	8.2%				

A fatty acid analysis of the total lipids was deemed essential to determine the level and the nature of unsaturated fatty acids present in this plant. These were investigated as methyl esters and the acids identified in the lipid mixture are presented in Table 3.

This accounts for more than 77 % of the unsaturated fatty acids in this plant, the highest being γ-linolenic acid, the route to eicosa-8,11,14-trienoic acid, the precursor of the prostaglandin PG_1 series. Until recently, arachidonic acid has been only rarely cited as a constituent of plant lipids (24). The presence of arachidonate (3.1 %) in *Aloe vera* lipids makes this plant unique in this respect. The occurrence of high yields of phosphatidyl choline and arachidonate in this plant is probably responsible for its efficacy in wound healing and cholera (4).

Table 4 Prostanoids produced by *Aloe vera* extracts when incubated with arachidonic acid.

Prostanoid	ng/g wet tissue	% Total Radioactivity
6-Keto-$PGF_1b\alpha$	0.93 + 0.24 (3)	5.03 + 1.24 (3)
$PGF_2\alpha$	1.89 + 0.28 (3)	10.36 + 1.21 (3)
TXB_2	3.69 + 0.87 (3)	19.23 + 1.40 (3)
PGE_2	10.16 + 2.45 (3)	52.66 + 3.52 (3)
PGD_2	2.22 + 0.79 (3)	11.80 + 3.62 (3)

After having established the presence of arachidonate, a potent precursor for the biosynthesis of prostanoids, we set out to establish the presence of cyclooxygenase in *Aloe vera*. It was of interest to determine the conversion of arachidonic acid to prostanoid synthesis. The presence of cyclooxygenase was demonstrated by the conversion of [^{14}C]-arachidonic acid into different prostanoids. The prostanoids identified by the TLC using authentic standards are shown in Table 4.

An incubation of the [^{14}C]-arachidonic acid with homogenized boiled plant resulted in complete inhibition of the prostanoid synthesis in the plant. Similarly an incubation of [^{14}C]-arachidonic acid with the homogenized plant in the presence of indomethacin did not produce any significant amounts of prostanoids. This confirmed the presence of cyclooxygenase in *Aloe vera* plant. An overall synthesis of PGE_2, a known bronchodilator (25), is much higher than that of $PGF_2\alpha$ and TXA_2 (stable metabolite TXB_2), bronchoconstrictor, in *Aloe vera*.

Shida and coworkers (14) have suggested that the enhancement of phagocytosis in adult bronchial asthma is due to the nondialyzable material from the pulp fraction of *Aloe* plant. However this activity was only exhibited if the plant extracts were kept in the dark at 4–30 °C for a period of three to ten days. These storage conditions are just right for the hydrolysis of phospholipids thus releasing arachidonic acid to synthesize prostanoids involving endogenously present cyclooxygenase as indicated by our results. Shida and coworkers (22) have not been able to identify the activity and have further reported that the plant extracts were not efficacious if the patient was pretreated with steroid drugs, a known inhibitor of phospholipase A_2.

The conversion of arachidonic acid to prostanoids by cyclooxygenase in a plant such as *Aloe vera* is a very interesting observation reported previously only in a few plants. The presence of endogenous prostaglandins in this plant is under investigation.

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