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Extraction and Identification of Bioactive Components from *Aloe barbadensis* Miller.

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Review Article

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ABSTRACT

Aloe vera is one of the oldest known medicinal plantbut it is now realized that many of its active constituent may be addressed in different ways by different formulations. Research since the 1986 review has largely upheld the therapeutic claims made in the earlier papers having scientific proof that *Aloe vera* contains constituents that accelerate wound healing, reduce inflammation, pain and itching. It has a wonderful moisturizing agent and penetrate.It was recently proven to stimulate the body's immune system and also has anti diabetic effect. There are many bioactive compounds present inaloe but there is need of an appropriate and standard method to extract these active components from plant material. Along with conventional methods, numerous new methods have been established but till now no single method is regarded as standard for extracting bioactive compounds from aloe. Several reputable suppliers produce a stabilized aloe gel for use as itself for formulations and there may be moves towards isolating and eventually providing verified active ingredients in dosable quantities. This review is aimed to discuss different extraction techniques for extracting bioactive compounds from *Aloe barbadensis* miller.

INTRODUCTION

Aloe vera L. (*Aloe barbadensis* Miller) is a member of Liliaceae family. Of over 300 Aloe species, *A. vera* L. is most widely accepted and used for various medical, cosmetic and nutraceutical purposes. Currently there is particular interest among the general public for medications that either are, or contain components, of natural origin. The true *Aloe vera* plant is called *Aloe barbadensis* Miller, otherwise called the Curacao aloe. *Aloe vera* (*Aloe barbadensis* Miller) is a perennial succulent cactus-like plant, which grows in hot, dry climates.[1]This plant is often referred to as a 'healing' plant and is the source of two products. The plant is made of elongated and pointed leaves .The first is an exudate from the cut leaf base which contains a high concentration of anthraquinone compounds and when dried is used as a potent cathartic and lacquer to inhibit nail biting. The second product, *Aloe vera*, is pressed from the whole leaf and is a clear mucilaginous gel possessing diverse putative pharmacological activity.[2]The inner gel from *A. vera* L., where most biological activity has been reported, contains natural polymeric carbohydrates. These natural carbohydrates consist primarily of -(1,4)-linked poly dispersed, highly acetylated Mannans with an average molecular weight of approximately 1000 kDa. The inner leaf gel consists of N98% water, whereas the acetylated Mannan moiety makes up greater than 60% of the solid matter found in the inner leaf after processing by precipitation with 95% alcohol and freeze drying.[3] This complex carbohydrate polymer is the material responsible for much of the immune stimulating activity attributed to the *A. vera* L. gel. [4]

The rich gel inside an Aloe plant is made up of over 75 different ingredients even though 95%-99% of it is water. These bioactive compounds have different pharmacological activity. All ingredients with their role are shown in table 1.

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Class	Compounds	Properties
Anthraquinones/anthrones	Aloe-emodin, aloetic-acid, anthranol, barbaloin, isobarbaloin , emodin, ester of cinnamic acid.	Aloin and emodin acts as analgesics, antibacterials and antivirals.
Carbohydrates	Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan, arabinogalactan, galactoglucoarabinomannan, pectic substance, xylan, cellulose	A glycoprotein with antiallergic properties, called alprogen and novel anti-inflammatory compound.
Chromones	8-C-glusoly-(2'-O-cinnamoly) -7-O-methlyaloediol A, 8-C-glucosyl-(S)-aloesol, 8-C-glucosyl-7-O-methylaloediol A, 8-C-glucosyl-7-0-methylaloediol, 8-C-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, neoalosin A	The novel anti-inflammatory commands .
Enzymes	Alkaline phosphatese, amylase,bradykinase, carboxypeptidase, catalase,cyclooxidase, cyclooxygenase,lipase, oxidase, phosphoenolpyruvate, carboxylase, superoxide dismutase	Bradykinase helps to reduce excessive inflammation when applied to the skin topically, while others help in the breakdown of sugars and fats.
Inoraganic compounds	Calsium,chlorine, chromium, copper,iron,magnesium, manganese,potassium,phosphorous, sodium,Zinc	They are essential for the proper functioning of various enzymes systems in different metabolic pathways and few are antioxidants
Miscellaneous including organic comounds and lipids	Arachidonic acid, Y-linolenic acid, steroids(campestrol, cholesterol, Bsitosterol), triglycerides, triterpenoid, gibberillin, lignins,potassium sorbate,salicylic acid, uric acid	
Proteins	Lectins, lectin-like substance	It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties. Lignin, an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients into skin. Saponins that are the soapy substances from about 3% of the gel and have cleansing and antiseptic properties.
Saccharides	Mannose, glucose, L-rhamnose, aldopentose	
Vitamins	Vitamin A, B12,C, E,choline and folic acid	Vitamin A, C and E are antioxidants and antioxidant neutralizes free radicals.
Hormones	Auxins and gibberellins	That helps in wound healing and have antiinflammatiory action.

Table 1: Composition and properties of *Aloe vera* gel [5,31,32,33,34,35]

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The present review is about isolation of these chemical constituents from *Aloe vera* gel. There are different methods described for different components.

BIOACTIVE CONSTITUENTS OF ALOE-VERA

Acemannan

Acemannan works in part by stimulating the macrophage, a key component of the immune system that is responsible for a wide range of potential health benefits. Acemannan has shown to accelerate wound healing.[6]Acemannan produces immune agents such as interferon and interleukin which help to destroy viruses, bacteria, and tumor (cancer) cells. Acemannan is a linear polysaccharide composed by (1,4)-linked mannosyl residues, with C2 or C3 acetylated and some side-chains formed by galactose units attached to C6. It is aβ-(1,4)-linkedpolydispersed, highly acetylated mannan with an average molecular weight of approximately 1000 kDa, is obtained from the inner leaf of *Aloe vera*. The structure of the acemannan monomeris shown below.

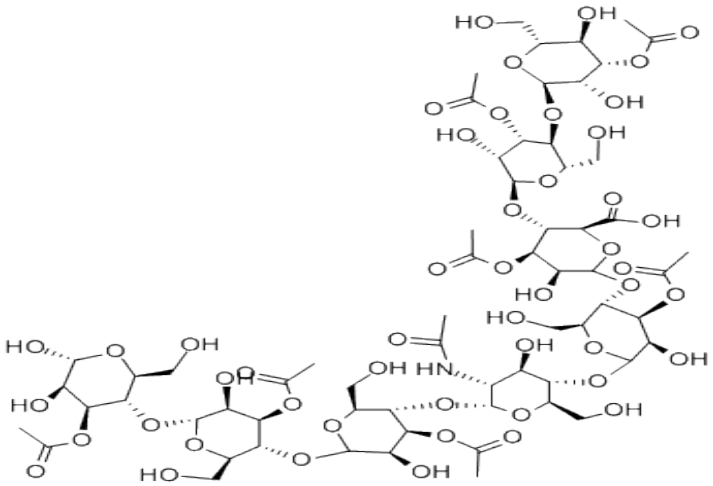

Figure 1: Structure of Acemannan[7]

Method of isolation

Solvent Extraction

The extraction of acemennan from *Aloe barbadensis* was done by solvent extraction method involved following steps. Collect approx 500 gm of *Aloe vera* gel fillets .Fill this gel fillets in a cotton cloth bag, according to soxhlet apparatus. At the bottom of the apparatus fill the solvent; ethanol (as acemannan is soluble in ethanol) 500 ml. Continue the extraction process for 48 hrs. After completion collect the extract and filter it and dry it. Weigh the amount of crude after drying and also measure the amount of aqueous extract. Store it for experimental analysis.[8]

Centrifugation

In this method leaves from several plantswere first allowed to drain off the yellow sap fromthe rind and then cleaned with deionized water.The rind was removed with a sharp blade. Theclear pulp was homogenized with a polytron. Thehomogenized pulp was centrifuged at 3000 rpm(500g, Beckman TJ-6) for 15 min. The pelletwas harvested and named pellet I. The supernatantwas further centrifuged at 18,000 rpm (25,000g;Beckman JA-20 rotor) for 30 min. The supernatantwas removed and saved. The new pellet wascollected and named pellet II. Both pellets werewashed once with deionized water, i.e., resuspending in deionized water and pelleted down at therespective centrifugation conditions. Pellets andsupernatant were then lyophilized (Centrivap, Labconco). The drying process was stopped whenvacuum level reached below 50 Am Hg. Theweight of dried materials was determined immediately after the drying process.The supernatant was also subjected to alcoholprecipitation by mixing with 3 volumes of 100%ethanol. The resulting precipitates were collected bycentrifugation and re-dissolved in water before being lyophilized as described above. Acemannan Hydrogel was similarly fractionedby centrifugation. It was first dissolved in water at 2mg/ml. The first centrifugation was performed at 1000rpm (180g) for 15 min. The pellets were washedwith deionized water as above.[9]

Purification of acemannan

There are different processes for purification of acemannan. Each is described below in detail.

Purification of acemannan was carried out through gel permeation chromatography [10]the elution of dialyzed fractions containing acemannan was performed on a column (100 cm £ 1 cm) of Sephacryl S-400-HR at a flow rate of 16 ml/hr. The fractions were dissolved in 2 ml, 50 Mm potassium–phosphate buffer, pH 6.5, containing 0.2 M NaCl. Fractions (2 ml) were collected and aliquots (20 ml) were assayed for carbohydrate by the phenol–sulphuric acid method. The appropriate fractions containing purified acemannan were combined, dialyzed, concentrated, and an aliquot was freeze dried for sugar and methylation analysis. The remaining material was stored at 220^{0}C.Analytical and preparative high-speed counter-current chromatography was successfully used for the isolation and purification of hydroxyanthraquinones from *Aloe vera* using pH-modulated stepwise elution.

Purification of acemannan by means of SEC and ethanolic precipitation

Aliquots with 200 mL of fi ltered supernatant were defrosted in a bathroom (IKA-Werk, Germany) at 37 ºC and then they were applied in a column packed with 300 mL of Sepharose® CL-4B gel. The running buffer solution was 0.2 M NaCl, pH 7; the flow used was 30 mL/min. The chromatographic separation process was monitored at 206 nm. For each chromatographic run, fractions coming from the first peak (corresponding to acemannan) were collected in volumes from 1 to 1.5 L. To the collected eluates, 95% (v/v) ethanol was added, at an ethanol:eluate proportion of 4:1 and then the mixture was kept at -20 °C for 6 h. The precipitate obtained was separated by centrifugation at 6700 x g for 20 min. The sediment was washed twice with 70% (v/v) ethanol, and vacuum dried. The dry material was stored at -20 °C in 50 mL Corning tubes until further resuspension. The resuspension was carried out in phosphate buffer solution (1.25 mg/mL $NaH_2PO_4{\cdot}2H_2O$ and 1.4 mg/mL $Na_2HPO_4{\cdot}\ 2H_2O$, pH 7.0 by means of agitation with a rotor-stator, until the polysaccharide was resuspended totally. Finally, this material was fi ltered with Minisart fi lters of 5 µm (pore average) size, to eliminate non-resuspended particles, and in this way to get a homogeneous solution. [11]

Purification of acemannan by using SEC, ultrafiltration and ethanolic precipitation

A volume of 3.5 L of collected eluates from the SEC run in the Process 1 was processed in a diafiltration system (model DC-2) from Amicon (Millipore, Billerica, MA, USA), equipped with hollow fiber cartridges of 30 kDa pore size operated at an entrance pressure of 0.7-1 bar. When the final volume of the material in the concentrator glass was of 1 L, the polysaccharide was then precipitated with ethanol as described above. [11]

Purification of acemannan by SEC, precipitation with cetyl trimethylammonium bromide (CTAB) and ethanolic precipitation

To 3.5 L of chromatographic eluates from the Process 1, 0.2 M sodium tetraborate salt (borax) was added until achieving a fi nal concentration of 0.01 M. The solution was maintained under agitation, at 24 ºC for 20 min. Subsequently, 10% (v/v) CTAB was added, until an appropriate concentration for the precipitation was reached. The resulting solution was retained under slow agitation at 4 °C for an hour. The precipitate obtained from the solution was separated by centrifugation at 8000 x g for 20 min. After that, 150 mL of 0.9 M CaCl2 solution was added and the mixture was homogenized with a rotor-stator. The final separation of the polysaccharide precipitated was carried out by adding 4 volumes of ethanol, at -20 °C for 6 h; this was collected by centrifugation at 8000 x g for 30 min.[11]

Purification of acemannan through direct precipitation with CTAB followed by ethanolic precipitation

The crude ethanolic material obtained, as described above, was weighed in the range from 3 to 30 g and resuspended in sodium tretaborate solution (0.01-0.1 M). The resulting solution was kept at 24 ºC under slow agitation for 20 min. Afterwards, a 10% CTAB solution was added and the mixture remained at 4 ºC under slow agitation for 1h. The precipitate attained was collected by centrifugation at 8000 x g for 20 min. To the resultant precipitate, 150 mL of 0.9 M $CaCl_2$was added, and the mixture homogenized .To the obtained total volume, 4 volumes of 95% ethanol were added, and the mixture was kept at -20 °C for 6 h. The polysaccharide precipitate was separated by centrifugation at 8000 x gfor 20 min. Next, two washes with 70% (v/v) ethanol were carried out, and the final material was dried off under vacuum. [11]

Aloin

Aloin is an anthraquinone glycoside. Aloin is also known as barbaloin. It has molecular weight 418, molecular formula $C_{21}H_{22}O_9$ and its chemicalstructure is shown in Fig. Its IUPAC name is 8-Dihydroxy-10-(□-D-glucopyranosyl)-3- hydroxymethyl) -9(10H)-anthracenone.[3]It is yellow-brown compound estimated at levels from 0.1 to 0.66 % of leaf dry present in cells adjacent tothe rind of the leaf in gel. It is used as laxative agent to maintain digestion system treating constipation by inducing bowelmovements. [12]Once ingested, it increases peristaltic contractions in the colon, and induces bowel movements. [14, 15]

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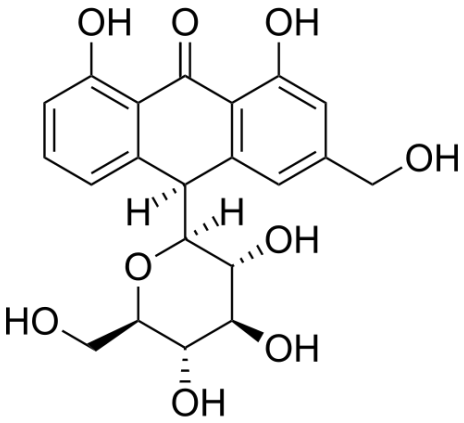

Figure 2: Structure of aloin. [36]

Method of isolation

Aloe vera leaves collected from local nursery . The leaves washed with water and rinds were removed. The inner gel scrapped and cut into pieces, solar dried (30-45 0C for 3 weeks) and dry gel particles were collected. The dry gel particles were screened using sieves in the range of 0.42-0.841, 0.841 to 1.68, 1.68- 3.36 & 3.36 – 6.73 mm respectively. Standard aloin sample was obtained from Fluka, USA for calibration. Solvents used for the extraction and high performance liquid chromatography (HPLC) analysis were of AR and HPLC grade from S. D. Fine Chemcials, India. [13]

Soxhelt extraction

The maximum recoverable aloin was estimated by Soxhelt extraction using methanol. 5 % (w/w) dry gel particles of size 0.42 – 0.841 mm were taken in Soxhelt with 200 ml methanol. Extraction was carried out for 24 hours. Samples free from dry gel were collected at the end, stored in a freezer, and analyzed using HPLC to determine the concentration of aloin in each extract.[13]

Batch extraction experiment

Batch extraction was conducted in a fully baffled 250 ml stirred borosilicate cylindrical glass vessel (7 cm ID, and 9 cm height) to estimate extraction kinetics and to analyze influence of the operating parameters. In a typical experiment, the vessel was charged with the dry gel along with 200 ml solvent. A volume of solvent in all experiment was taken 200 ml and dry gel loading changed accordingly. A four pitch bladed 450 turbine agitator of 3.5 cm diameter was used to stir the mass at a predetermined rpm. All experiments were conducted in a thermostatic bath. The temperature of system was measured with accuracy of 1 0C. Samples were collected at different time intervals for entire duration of the extraction and analyzed by HPLC to estimate the concentration of aloin. At the end of each experiment, the solution was filtered and volume was adjusted at its initial value to avoid errors in the aloin concentration due to vaporization loss. The effect of solvent of varying polarity (e.g. methanol, ethanol, water, and IPA), speed of agitation (300,600,900, and 1200 rpm), particle size (0.42-0.841, 0.841- 1.68, 1.68- 3.36, 3.36-6.73 mm), solid loading (2.5 %, 5 %, 7.5 %, and 10 %) and temperature (30 0C, 40 0C, 50 0C, 600C and 65 0C) were investigated to select optimum conditions for the batch extraction.[13]

Ultrasound assisted extraction method (UAE)

It intensifies the kinetic of the extraction process by acting upon the interfacial area, through the disintegration of particles. Compared with batch extraction, it improves the extraction process decreasing both extraction time and temperature while increasing the rate of extraction. UAE technique was used to obtain active principle aloin from a herbal plant *Aloe vera*. Methanol was selected as organic solvent as found maximum extraction of aloin with it. The active principle aloin was quantified using WATER's HPLC system. The optimum extraction conditions were estimated as extraction temperature, extraction time and dry gel loading. Effective intra particle diffusivity of aloin in methanol solvent was estimated using unsteady state mass diffusion model, and activation energy of diffusion using Arhenius equation respectively.[17]

Identification of aloin

Identification of aloin can be done by different chromatographic techniques. L. Azaroualet. al. used this techniques and it was achieved by comparison of retention times and UV spectra of separated compounds as well as by coelution with the only authentic standard available (aloin A). Additionally HPLC-MS analyses were also

carried out.A Finnigan LCQ-coupled LC-MS system was used for the HPLC-MS analyses of extracts. This equipment is fitted with a Spectra SYSTEM 2000 model gradient pump and a mass detector (model LCQ), consisting of an electrospray interface and an ion trap mass analyzer. Xcalibur, version 1.2, was the software used for the control of the equipment, and the acquisition and treatment of data. The sample injection volume was 25 µL. Interface conditions: positive ionization, temperature of the capillary: 220.C, spray voltage: 20 kV, capillary voltage: .5V, sheath gas flow: 80 (arbitrary units), and auxiliary gas flow: 10 (arbitrary units). API-MS spectra were acquired in the m/z range of 50.400. The chromatographic method used was a gradient elution, using acidified water (2% acetic acid, solvent A) and acidified methanol (2% acetic acid, solvent B), at a flow rate of 0.2mL/min. The gradient employed was as follows: 0 min, 0% B; 1min, 0% B; 5min, 30% B; 20 min, 30% B; 30 min, 50% B; 40 min, 100% B. A C-18 column (Luna 5 µm, 150 × 3 mm, Phenomenex) was used for the chromatographic separation. [30]

Aloe emodin

Aloe emodinis ananthraquinone present in aloe latex, an exudate from the aloe plant. [10]Aloe-Emodin was able to inhibitcell growth in several tumor cells, including human lung carcinoma, [18]hepatoma, [19]and leukemia celllines.[20]One of the polysaccharide present in *Aloe vera*, Acemannan, is known for its ability to restore andboost the immune system by stimulating the production of macrophages and improving the activity of TLymphocytesby up to 50 %.

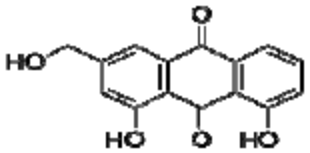

Figure 3: Structure of emodin[16]

Method of isolation

Fresh whole *Aloe vera*barbadensis miller leaves collected from medical garden. The leaves are of between 30 and 50 cm of length,corresponded to 4-year old plants. Whole leaves were washed with distilled water to remove dirt from thesurface. The spikes, placed along their margins, were removed before slicing the leaf. The epidermis (orskin) was carefully separated from the parenchyma using a scalpel-shaped knife. The filets wereextensively washed with distilled water to remove the exudates from their surfaces. Fresh aloe filets werestored no longer than 1 hr at 180C.[16]

Dehydration

Fresh aloe filets were diced to 0.5 mm 3 cubes, and dehydrated in a hot air oven. Temperatures used were 30 ^{0}C (30D sample), 40 ^{0}C (40DSample), 50 ^{0}C (50D sample), 60^{0}C (60Dsample), 70 ^{0}C (70D sample) and 80^{0}C(80D sample).Cell wall polysaccharides comprised mainly ofpectic substances, cellulose, and also hemicelluloses. Cellulose was the second cell wall polymer type inabundance. Final moisture content of the dried parenchyma cubes was 1.5 g water / 100 g dry matter (d.m.).In addition, an aloe filet was freeze dried (FD sample), and used as a reference. [21]

Identication of emodin

Identification of emodin done by ultraviolet spectrophotometric method of analysis: The graph was plotted between absorbance and wavelength of radiation. The range detected was between 222 -432. The three absorbtion maxima are obtained at 223, 256, 283 wavelength. Ultraviolet analysis revealed the structure of emodin from the dehydrated filets. The structure of emodin reveals anthraquinone ring having three hydroxyl group and two ketonicgroups. The three hydroxyl group attached at 1, 3 and 8 position. [16]

Lignin

It a woody substance, helps in a penetration of *Aloe vera* gel.

Method of isolation

Lignin was gravimetrically determined as Klason lignin(alcohol insolubleresidue) AIRs were dispersed in 72% H2SO4 at room temperature for 3 hrs then diluted to 1 M H2SO4 andheated to 100^{0}C for 2.5 hrs. Insoluble material was recovered by filtration (sinter no. 2) and washedthoroughly with hot water (90^{0}C) until acid free before drying at 105^{0}C overnight. The residue weight was recorded as Klasonlignin, Ash contents were gravimetrically determined by overnight heating at 550^{0}C, Carbohydrate analysis was performed [22]for neutral sugars. Sugars were released from residues by acidhydrolysis.[23]Iron and phosphate were determined by colorimetric methods of

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analysis, Vitamin c wasdetermined by volumetric methods of analysis, identification of acemannan was done by FTIR .Fouriertransformed infrared (FTIR) spectra were obtained at a resolution of 3 cm^2, after preparing a KBr disccontaining 2 mg of purified acemannan polymer from the freeze dried and dehydrated aloe filets. The single beam transversing each sample was ratio with the single beam of the corresponding background.Equivalent samples from different experimental runs gave the same spectra in all cases. [24]

Isoaleoresin D

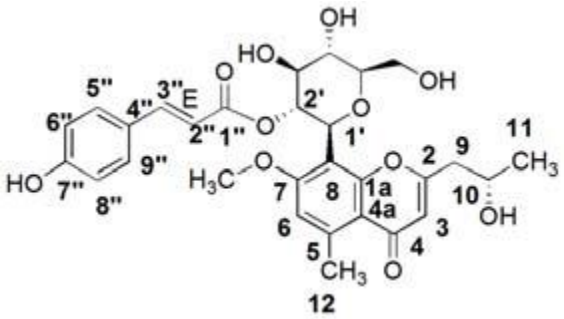

Figure 4: Structure of isoaleoresin D

Extraction of isoaleoresin D

Aloe powder (25.0 g) was extracted with 500 mL of acetone by sonication for 30 min, and then centrifuged. The supernatant was concentrated under the reduced pressure at 60 in a rotary evaporator. The slurry was dried in vacuum at 60. Extract (16.3 g) was obtained and stored under below 10for the High-speed counter-current chromatography (HSCCC) separation and isolation. Acetone extract (513.0 mg) was dissolved by methanol and transferred to a 10 mL volumetric flask. The solution was diluted with four times of methanol and the concentration was 12.8 mg/mL. The solutions were filtered through a 0.45 μm membrane filter before injected into HPLC. [26]

Isolation and purification

The biphasic solvent system consisting of hexaneethyl acetate-acetone-water (0.2 ∶ 5 ∶ 1.5 ∶ 5) was applied for the HSCCC separation and isolation. The solvent mixture was thoroughly equilibrated in a separatory funnel at ambient temperature. The two phases were separated and degassed by sonication. The preparative column of HSCCC was entirely filled with the lower aqueous phase as stationary phase and the elute was conducted in tail-to-head mode. Then the upper organic phase was pumped at a flow rate of 1.0 mL/min while the apparatus was rotated at 840 r/min. The aloe extract was dissolved with lower aqueous phase, of which the concentration was 85.5 mg/mL. After the mobile phase front emerged and the system had reached hydrodynamic equilibrium, sample solution (4.5 mL) was injected into the column. The stationary phase retentionwas 79.1%. The effluent was monitored at 254 nm and the fractions were collected at the intervals of 5 min. The total time of HSCCC separation was 320 min. The fractions were combined according to the chromatograms of the HSCCC and HPLC. As the result, the fraction 1 was collected from 99 to 144 min, while the fraction 2 was collected from 152 to 250 min. [26]

Steriods

Campesterol, β-sitosterol and cholesterol.[36]

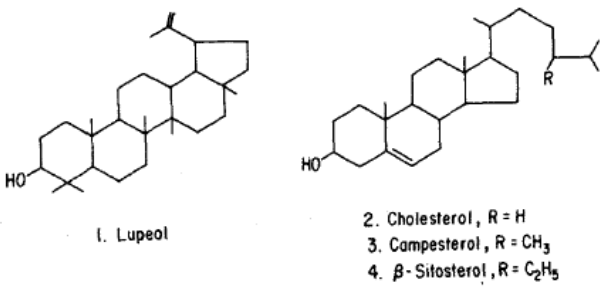

Figure 6: Stucture of triterpenoid and sterols in aloe[25]

Campesterol is a phytosterol whose chemical structure is similar to that of cholesterol. It is the precursor of anabolic steroid boldenone which is commonly used in veterinary medicines to induce growth in cattle [28]. It was found beneficial in lowering LDLs and cholesterol. It is thought that the campesterol molecules compete with cholesterol and thus reduces the absorption of cholesterol in the human intestine [29].Cholesterol, campesterol, β-sitosterol, and lupeol were found in substantial amounts inthe lipid fraction. An unknown(s) alkaloid was detected using Dragendorff's reagent.

Extraction and fractionation

Since there were reports that severed Aloe leaves rapidly lose their medicinal properties the material used was either fresh or lyophilized and stored at −15 C. A. barbadensis leaves (35 g equivalent of dry material) were macerated and extracted with water-acetone (1:1) and then with acetone at room temperature. The combined extracts (2:1) were concentrated in a rotary evaporator (35 C) and the acetone-free residue was extracted three times with diethyl ether (250 ml each time). The lipid phase was taken to dryness and the residue refluxed with 10% potassium hydroxide in 50% ethanol (50 ml) for 3 hr. This hydrolysate was extracted with diethyl ether and the concentrated organic phase (0.25 g) was chromatographed on a silica gel column (1.5 × 35 cm), eluted with benzene: diethyl ether (8:2), and collected in 10-ml fractions. The resulting 20 fractions were assayed by thin-layer chromatography (TLC) (see chromatography section74: silica gel, solvent 4, chromogenic reagent b) and recombined to give triterpenoid and sterol fractions. The aqueous phase (250 ml) was concentrated under reduced pressure, clarified by centrifugation, and passed successively through Dowex-50 W (50-100 mesh, × 8, H+) (3.5 × 35 cm) and Amberlite IRA-400 (50-100 mesh, × 8, OH−) (4 × 40 cm) columns. The aqueous eluate was taken to dryness and constituted the "neutral fraction." The "cationic fraction" was obtained by treatment of the cation exchange resin with 2N NH4OH.[27]

Steam distillation

The tough outer portion consisting of the cuticle, epidermis, and mesophyll was removed from the leaves of mature plants brought from Texas. The green outer portion and the colorless inner part as well as the "stalk" (that portion of the plant above the ground remaining after the leaves had been removed) and the roots were steam distilled, each separately. The distillation was allowed to proceed as long as the condensate had a definite odor; this was approximately 5 hr. The condensate from the steam distillation was saturated with sodium chloride, extracted with diethyl ether, dried with anhydrous sodium sulfate and the ether was reduced in volume using a stream of nitrogen.[27]

Chromatography and mass spectrometry

Precoated silica gel plates were used for TLC. The solvent systems were: 1) 1-butanol:aceticacid:water (12:3:5); 2) phenol:water (3:1); 3) 1-butanol:acetone:water (7:2:1); 4) benzene:diethylether (8:2); 5) chloroform:methanol (5:1); 6) hexane:acetone:ethanol (40:10:4). Solvents 1 and 2 were used in that order to develop two-dimensional thin-layer chromatography (2-D TLC). Chromogenic reagents were: 0.1% ninhydrin in acetone; b) 1% ceric sulfate Ce(SO4) 2 in 2N H2 SO4; c) 0.05% Rhodamine 6G in acetone; d) Dragendorff's reagent (25). GLC was performed using a modified Barber Colman Model 5000 gas chromatograph with flame ionization detector (30) and He as carrier gas. The following columns were used: I) 3 m × 4 mm glass column packed with 4% OV-101 on 100-110 mesh Anakrom ABS; II) 2 m × 4 mm glass column packed with 3% SE-30 on 80-100 mesh Chromosorb W (HP); III) 1.5 m × 4 mm glass column packed with 3% OV-25 on 80-100 mesh Chromosorb W (HP). A prototype of the LKB-9000 was used for GLC-MS (26). A Beckman amino acid analyzer, Model 120C (AAA), was utilized for quantitative determination.[27]

Examination of fractions

Cationic fraction

Amino acids present in this fraction were identified and quantitated by 2-dimensional TLC and by AAA, conjunctively. Glutamine and asparagine were determined by measuring the increase of parent acids following hydrolysis (1N HCl /3 hr in N2). Neutral fraction. A dry aliquot (10−2) of this fraction was trimethylsilylated (TMS). In order to avoid tailing, the reagents were removed before GLC by extraction with chloroform. Column I was used isothermally at 190 C, flow rate 50 ml/min. Peaks were identified by comparison with standards as D-mannose (one peak) and D-glucose (two peaks). Results were confirmed by TLC of the free sugars (silica gel, solvent 3, chromogenic reagent b). Determination of both TMS-sugars (whose molar detector constants were experimentally found to be 1.0) was achieved using α-D-xylose as an internal standard. The peak area was calculated triangulation (retention time relative to that of TMS-α-D-xylose : TMS-α-D-mannose 1.38; TMS-α-D-glucose 1.78; TMS-β-D-glucose 2.35).

A sample of the transparent gel-like portion of the Aloe leaf which had been freed of all the green material was lyophilized and a sugar analysis was performed. The lyophilized sample was hydrolyzed in 2N H2SO4 for four hr at 100 C. The hexosamines were separated from the neutral sugars on a Dowex H+ column and the neutral sugars were determined by the method of Lee, McKelvy, and Lang.[27]

Steam distillation

The blended gel changed from nearly colorless to a very deep pink during distillation. The color change was not due to a change in pH, for the pH remained at 5.0 during the distillation. Then the distillation mixture was made acidic to pH 1.0. Following the aciddistillation, the gel was made basic with 10% aqueous sodium hydroxide and distillation was continued. The gel became very dark in color and appeared to be partly digested by the base, while the distillate had an odor similar to that of burnt wood. The Dragendorff's positive reaction indicated that Aloe contains at least one alkaloid.Repeated attempts to extract material that gave a Dragendorff's positive reaction were made but nopositive results were obtained. After spraying with Dragendorff's reagent, some compounds present in a methanol extract or a dichloromethane extract at the origin of the TLC plate (solvents 5 and 6) showed a slowly developing blue coloration. This may mean that the Aloe develops a light-sensitive compound when sprayed with Dragendorff's reagent or it may contain a complex alkaloid that is set free upon distillation.

Triterpenoid fraction

Fraction 3 to 5 were chromatographed by preparative TLC (silica gel, solvent 4, chromogenic reagent b). The band with Rf 0.53 was eluted with diethyl ether; this gave 12 mg of a mixture, the major component (96%) of which was identified as lupeol by NMR and GLC-MS (Column III,operating isothermally at 220° C, flow rate 30 ml/min). The major mass spectral fragments agree with thestandard values observed and reported. [30, 31] The NMR spectrum proved particularly informative with respect to the isopropenyl side chain in ring E. The methyl group at C-29 appeared as a singlet at δ 1.66.[17] The two olefinic protons resonated at 4.56 and 4.68 δ (Jgem 1.8 Hz).

Sterol fraction

The sterols were in fractions 7 to 15. Crystallization from methanol gave 30 mg of amixture with m.p. 132-134 C. The IR spectrum (KBr) gave strong absorption bands at γ max. 3400, 2930, 1460, 1375, 1060, 1025 cm–1, GLC-MS (column II isothermally at 245 C, flow rate 30 ml/min) showed the mixture to be composed of cholesterol (M+386) (7%), campesterol (M+400) (6%) and (M+414) (87%). The spectra were consistent with those of standard samples and with the published fragmentation patterns of sterols.[27]

CONCLUDING REMARK

Due to the growing demand for Plant bioactive compounds, there is the need for search of convenient extraction method. It is important to consider that these extractions are based on different mechanisms and extraction efficiency varies process vise. The available experimental data is also not sufficient for high yield extraction. Currently, however, problems such as lack of consistency and yield seem to be overshadowing the potential genuine health benefits of aloe plant.Thus, the increasing economic significance of these bioactive compounds may lead to find out more sophisticated extraction method in future.

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