

IDENTIFICATION OF PHYTO-COMPONENTS AND ITS BIOLOGICAL ACTIVITIES OF *ALOE VERA* THROUGH THE GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT

In this study, the bioactive components of *Aloe vera* leaves have been evaluated using GC/MS. The chemical compositions of the n-hexane extract of *Aloe vera* were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of n-hexane extract of *Aloe vera* revealed the existence of twenty six bioactive compounds. The results of this study offer a platform of using *Aloe vera* as herbal drug for cancer studies.

KEYWORDS: *Aloe vera*, GC-MS, cancer, n-hexane, medicinal plant

INTRODUCTION

Plants possess components, which render beneficial properties¹. Hence, currently attention is being drawn towards exploring plant sources for substances that provide nutritional and pharmaceutical advantages to humans. Use of plants as a source of medicine has been inherited and is an important component of the health care system². Natural products or plants derived compounds contribute to a great extent in fight against cancer. The biological inhibitions by different natural substances, such as essential oils and plant extracts have been investigated widely against cancer³. Since olden days, plants are used to treat many ailments. India has about 45,000 plant species and several thousands have been claimed to possess medicinal properties⁴.

Aloe vera is an important medicinal plant that belongs to the family Liliaceae⁵. *Aloe vera* is a stem less or very short-stemmed succulent plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on the upper and lower stem surfaces. The margin of the leaf is serrated and has small white teeth. The flowers are produced on a spike up to 90 cm (35 in) tall, with a red tubular corolla 2–3 cm (0.8–1.2 in) long. *Aloe vera* is a succulent from the *Aloe* family (400 different species) with its origin in African continent. Its thick leaves contain the water supply for the plant to survive long periods of drought. The leaves have a high capacity of retaining water also in very warm dry climates and therefore this plant can survive very

harsh circumstances where most other vegetation disappears. When a leaf is cut, an orange-yellow sap drips from the open end. When the green skin of a leaf is removed a clear mucilaginous substance appears that contains fibers, water and the ingredient to retain the water in the leaf. This is called the gel. *Aloe vera* gel consists of 99.3% water. The remaining 0.7% is made up of solids with glucose and mannose constituting for a large part. These sugars together with the enzymes and amino acids in the gel give the special properties as a skin care product⁶.

Aloe vera is an ancient medicinal plant whose anti-inflammatory⁷, wound-healing⁸, antidiabetic⁵, antimicrobial⁶, antifungal³, antioxidant⁵, anticancer⁹, antineoplastic¹⁰ and immune system enhancers¹¹ qualities have attracted significant scientific interest. The research has shown that the compounds and constituents of *Aloe vera* play a vital role in the prevention and control of numerous diseases.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh shoots of *Aloe vera* were collected from uncultivated farmlands located under the foothills of Marudhamalai hills, Coimbatore in the month of December 2007. The whole plant material was washed thoroughly under running tap water and rinsed with distilled water, and dried with blotting paper, kept under shade for drying and cut into small pieces dried and made into fine powder. The powder of the plant material was used for extraction.

Extraction of Active Compounds from the Whole of *Aloe Vera* Leaves

Grind 100 g dried *Aloe vera* with occasional shaking for 1 to 2 min. Weigh exactly 10 g of sample into cellulose extraction thimbles. Cover the top of each thimble with glass wool to prevent floating. Weigh the pre-dried flat-bottom extraction flask with a few boiling chips or glass beads. Extract the active compounds in *Aloe vera* with 150 ml of hexane at the boiling point for 3 to 6 h in a Soxhlet extractor¹² using a heating mantle. The boiling point of hexane is 69°C. The condensation rate for the solvent was set at 2 to 6 drops per second. The extractives were collected, filtered and extracted in an ash less filter paper with sodium sulphate [2gm] and concentrated the extract to 1ml¹³. The extract was used for GC-MS analysis.

Sample Preparation and Conditions for Gas Chromatography – Mass Spectrometry (GC–MS)

GC/MS analysis of the *Aloe vera* extract was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with an Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 µMdf composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the injection volume of 2 µl was employed (split ratio of 10:1). Injector temperature was 250°C and Ion-source temperature was 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver5.2.0.

RESULTS AND DISCUSSION

Twenty six compounds were identified in *Aloe vera* leaf extract by GC-MS analysis. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and Concentration (%) are presented in Table 1. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

Ten compounds with its biological activities were found in *Aloe vera* (Table 2). The prevailing compounds were n-Hexadecanoic acid(20.47%), Oleic Acid(14.53%), 1,2-Benzenedicarboxylic acid, diisooctyl ester(13.60 %), Squalene(6.60%), 1-Heptanol, 2-propyl-(3.82%), 1,2-Benzenedicarboxylic acid, butyl octyl ester (2.30 %), Tetradecanoic acid (1.04%), the compounds were found within the retention time span between 10 - 40 min. The utilization of GC-MS was effective for the identification of the bioactive compounds from *Aloe vera*. Major compounds in *Aloe vera* were shown to have the activity as anticancer, antimicrobial etc.; the composition of identified active principles in *Aloe vera* has been the subject of future research studies.

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Table 1. Total ionic chromatogram (GC–MS) of hexane extract of *Aloe vera* obtained with 70 eV using an Elite-1 fused silica capillary column with Helium gas as the carrier.

No	RT	Name of the compound	Molecular formula	MW	Peak Area %
1	3.06	p-Xylene	C ₈ H ₁₀	106	3.18
2	3.78	1,5-Heptadien-4-one, 3,3,6-trimethyl-	C ₁₀ H ₁₆ O	152	1.74
3	5.44	Undecane	C ₁₁ H ₂₄	156	0.46
4	7.03	1-Heptanol, 2-propyl-	C ₁₀ H ₂₂ O	158	3.82
5	8.25	Tridecane	C ₁₃ H ₂₈	184	0.14
6	9.47	7-Tetradecene, (Z)-	C ₁₄ H ₂₈	196	0.22
7	10.87	Tetradecane	C ₁₄ H ₃₀	198	0.37
8	12.14	Hexadecane	C ₁₆ H ₃₄	226	0.43
9	13.64	12,15-Octadecadiynoic acid, methyl ester	C ₁₉ H ₃₀ O ₂	290	0.21
10	13.96	(4,7-Dinitronaphthalen-1-yl)-(4-methoxyphenyl)diazene	C ₁₇ H ₁₂ N ₄ O ₅	352	0.08
11	14.44	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	1.04
12	14.93	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366	0.23
13	15.14	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	C ₂₈ H ₄₄ O ₄	444	0.08
14	16.58	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	C ₂₇ H ₅₂ O ₄ Si ₂	496	0.10
15	16.73	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	0.47
16	17.28	1,2-Benzenedicarboxylic acid, butyl octyl ester	C ₂₀ H ₃₀ O ₄	334	2.30
17	17.42	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	20.47
18	19.38	11,14-Eicosadienoic acid, methyl ester	C ₂₁ H ₃₈ O ₂	322	0.70
19	19.47	1-Monolinoleoylglycerol trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	498	0.67
20	20.22	Oleic Acid	C ₁₈ H ₃₄ O ₂	282	14.53
21	26.29	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	390	13.60
22	27.15	Eicosane	C ₂₀ H ₄₂	282	3.38
23	28.71	Heptacosane	C ₂₇ H ₅₆	380	6.11
24	29.07	Octacosane	C ₂₈ H ₅₈	394	9.56
25	31.20	Squalene	C ₃₀ H ₅₀	410	6.60
26	35.43	Hentriacontane	C ₃₁ H ₆₄	436	8.18

Table 2. Major Phyto-components and its biological activities obtained through the GC/MS Study of *Aloe vera* have been listed

No	RT	Name of the compound	Peak Area %	Activity
1	7.03	1-Heptanol, 2-propyl-	3.82	Antimicrobial
2	13.96	(4,7-Dinitronaphthalen-1-yl)-(4-methoxyphenyl)diazene	0.08	Antimicrobial
3	14.44	Tetradecanoic acid	1.04	Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic
4	16.73	Hexadecanoic acid, methyl ester	0.47	Antioxidant, Nematicide, Hypocholesterolemic, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic
5	17.28	1,2-Benzenedicarboxylic acid, butyl octyl ester	2.30	Antimicrobial, Antifouling
6	17.42	n-Hexadecanoic acid	20.47	Antioxidant, Nematicide, Hypocholesterolemic Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic
7	19.38	11,14-Eicosadienoic acid, methyl ester	0.70	Hypocholesterolemic
8	20.22	Oleic Acid	14.53	Anti-inflammatory, Antiandrogenic, Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic, Insectifuge, Flavor
9	26.29	1,2-Benzenedicarboxylic acid, diisooctyl ester	13.60	Antimicrobial, Antifouling
10	31.20	Squalene	6.60	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Pesticide Immunostimulant, Chemo preventive, Lipoxigenase-inhibitor

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