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A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC PHYTOCHEMICAL ANALYSIS OF LYOPHILIZED GEL FROM *ALOE BARBADENSIS* LEAVES

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ABSTRACT **Background:** Aloe vera not used extensively in cosmetics but also proven to have beneficial medicinal properties for skin diseases, as laxative, in colitis and has antimicrobial properties. Phytochemical composition of Aloe vera is not so well studies till now especially with modern analytical techniques. **Material and Methods:** Lyophilized gel of Aloe vera leaves succulent gel was studied. Aqueous and methanolic extracts of Lyophilized Aloe vera succulent gel (AVS) were studied separately. HPLC machine and data acquisition system (Young Lin Instrument Co., Ltd., Korea) was used to perform HPLC analysis of both of the extractsA basic chemical analysis was also performed for present/absence of most common phytochemicals to compare with HPLC results. **Results:** The results of initial chemical analysis for phytochemical characterization revealed presence of Flavonoids, Saponins, Tannins, Reducing sugars, Glycosides, Starch and Sterols, While test for Alkaloids, Cardiac glycosides, Triterpinoids and Coumarins showed negative results. There were total 7 peaks identified in HPLC chromatogram for aqueous extract with retention time for last compound (peak) for aqueous extract was 7.59 minute andhighest peak height found to be was 27.6675 mV with corresponding Retention time (RT) of 4.8167 min. There were total 14 peaks were identified in HPLC chromatogram for methanolic extract with retention time for last compound (peak) for methanolic extract was 13.37 minute and highest peak height found to be was 166.1689 mV with corresponding Retention time (RT) of 6.1050 min. **Conclusion:** Analysis reveal presence of many phytochemical compound such as Anthraquinone, flavonoids, alkaloids, tannins, phenols, -sitosterols etc in the inner succulent gel of *Aloe vera*.

KEYWORDS : HPLC, Phytochemical, Aloe vera

INTRODUCTION

Development of new drug is a very complex, time-consuming, and expensive process. Development of a single agent usually take an average of 12 years and 1 billion US dollar for identification of identification of suitable new chemical entities (NCEs), either sourced through chemical synthesis or through isolation from natural products. This approach of chemical, however, was proven to be less effective. The second source of NCEs for potential use as drug molecules has been the natural products. Before the advent of high throughput screening and the post genomic era, more than 80% of drug substances were purely natural products or were inspired by the molecules derived from natural sources (including semi-synthetic analogs).(1) One such important medicinal plant is *Aloe vera* not used extensively in cosmetics but also proven to have beneficial medicinal properties for skin diseases, as laxative, in colitis and has antimicrobial properties.–(2)

High Performance Liquid Chromatography (HPLC) is a versatile, robust, and widely used chromatographic technique for the isolation of natural products, which can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture.(3) There are hundreds reported constituents found in the leaves and in the gels. Aloe pulp has been shown to contain protein, lipids, amino acids, vitamins, enzymes, carbohydrates and inorganic compounds. The yellow exudates contains 1,8-dihydroxyanthraquinones and their glycosides. Aloin, an anthraquinone-C-glycoside, naturally occurs as a mixture of diastereoisomers. The amount of aloin is also extremely variable among different species and highly depends on the growing conditions. The concentrations of these phytochemicals also depend on the location of the leaf within the aloe plant as well as the site within the leaf, i.e., leaf margins, pulp center, etc.(4)

This HPLC analysis was carried out on Lyophiized Aloe vera (*Aloe Barbadensis*) gel to characterized the phytochemical composition of locally occurring Aloe vera plantation.

MATERIALS AND METHODS

Aloe vera leaves for present study were collected from plant purchased from **Haryali nursery** situated at **Palace road, Gwalior.** Lyophilized gel of *Aloe vera* leaves succulent prepared at Microbiology Department of Defense Research and Development Establishment, Gwalior. Distilled water-HPLC grade (Ranbaxy lab. Ltd. Mumbai) was used to prepare aqueous extract Lyophilized Aloe vera succulent gel (AVS). Similarly Methanol- HPLC grade (Ranbaxy lab Ltd. Mumbai) was used to produced methanolic extract of the same. Vacuum filter and Sonicator was used to clear any particulate material from both of the extract before injecting it for analysis. HPLC machine and data acquisition system (Young Lin Instrument Co., Ltd., Korea) was used to perform HPLC analysis of both of the extracts. Since standard plots were not available on the system results from other studies were used to comment on results of the Chromatogram obtained. A basic chemical analysis was also performed for present/absence of most common phytochemicals to compare with HPLC results.(5)

Preparation of extract of *Aloe vera*

This study was conducted as a part of thesis project for M.D. (Pharmacology) at Department of Pharmacology, Gajara Raja Medical College, Gwalior in 2012-13. Interpretation of results was performed at Department of biochemistry and Pharmacology in year 2020 at Netaji Shubhas Chandra Bose Medical College, Jabalpur. The leaves of *Aloe vera* plant were obtained from **Haryali nursery, Palace road, Gwalior**. The collected leaves were washed in cold water. Skin (rind) of leaves was separated carefully from inner succulent with the help of knife. The succulent was cut into small pieces and grind in mixture till a gel like material was obtained which is filtered through muslin cloth. This material weighed and 2 kg of it taken into air sealed jars and immediately transported to Microbiology Department of Defense Research and Development Establishment, Gwalior. At DRDE the material

was lyophilized for 7 days and a light yellow dry powder was produced. This lyophilized *Aloe vera* succulent gel was weighted and yield was found to be 0.3%. This Lyophilized *Aloe vera* succulent (AVS) was used as fresh made suspension in various experiment.

HPLC Analysis of AVS

Principle

HPLC work on basic chromatography principle of relative difference in relative affinities to stationary and mobile phase of different molecules in a mixture leads to the separation. The difference is that it is sophisticated instrument with high accuracy to form peaks in chromatogram of absorption. Retention time, height and area can be measure with the help of software and data can be used to detect number, quality and also quantity of particular constituent in sample.

Equipment

HPLC analyzer of Young Lin was used, which has YL 9111 binary pump system along with YL 9160 PDA detector. HPLC machine connected to a computer system installed with data acquisition system Autochrom 3000 software (verson 2.0.4 of Young Lin Instrument Co., Ltd., Korea).

Procedure

Fifty milligram of AVS is kept in 5 ml of methanol and distilled water for 24 hrs to get soaked and then centrifuged at 5000 rpm for 5 min. supernatant is filtered through Whatman filter paper no. 1. Filterate was sonicated for 10 min and transferred to labeled test tubes as sample. HPLC started and after purging and setting flow rate at 1 ml/min. HPLC grade methanol and water were used in 50:50 as mobile phase. After injecting and load respective sample of methnolic and aqueous extracts of AVS, chromatograms were acquired at 254 nm.

OBSERVATIONS AND RESULTS

The results of initial chemical analysis for phytochemical characterization revealed presence of Flavonoids, Saponins, Tannins, Reducing sugars, Glycosides, Starch and Sterols, While test for Alkaloids, Cardiac glycosides, Triterpinoids and Coumarins showed negative results.

Table 1: Integration values for Aqueous extract of AVS

Peaks	RT[min]	Area%	Height[mV]	Height%
1	3.6617	6.32	3.7793	5.58
2	4.8167	55.20	27.6675	40.83
3	5.5450	7.91	7.0247	10.37
4	5.9150	3.45	6.8003	10.04
5	6.1450	8.80	6.0224	8.89
6	6.8483	7.95	8.6945	12.83
7	7.5950	10.38	7.7674	11.46
Sum			**67.7561**	

There were total 7 peaks identified in HPLC chromatogram for aqueous extract of lyophilized succulent of Aloe vera leaf. The retention time for last compound (peak) for aqueous extract was 7.59 minute. The highest peak height found to be was 27.6675 mV with corresponding Retention time (RT) of 4.8167 min followed by peaks of 12.83 and 11.46 mV at 6.8483 and 7.5950min respectively.

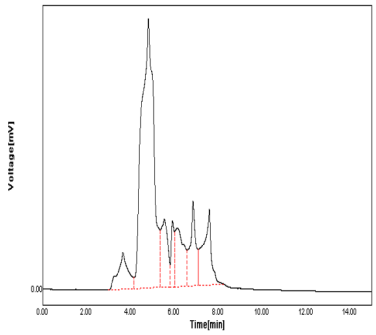

Figure 1 : Chromatogram For aqueous extract

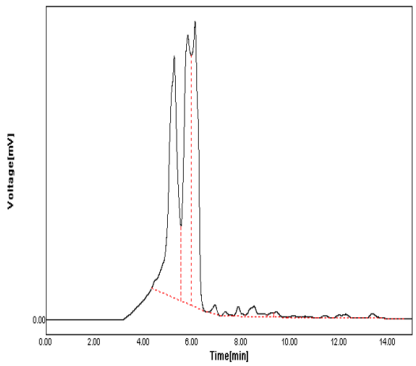

Figure 2: Chromatogram for methanolic extract

There were total 14 peaks were identified in HPLC chromatogram for methanolic extract of lyophilized succulent of Aloe vera leaf. The retention time for last compound (peak) for methanolic extract was 13.37 minute. The highest peak height found to be was 166.1689 mV with corresponding Retention time (RT) of 6.1050min followed by peaks of 156.2899 and 140.8054 mV at 5.8083 and 5.2533min respectively. Highest percent area is seen with the peak of 140.8054 mV at RT of 5.2533min.

Table 2: Integration values for methanolic extract of AVS

Peaks	RT [min]	Area%	Height [mV]	Height%
1	5.2533	35.36	140.8054	28.34
2	5.8083	30.43	156.2899	31.46
3	6.1050	28.29	166.1689	33.44
4	6.9167	0.69	5.3685	1.08
5	7.3467	0.36	2.6996	0.54
6	7.8700	0.71	5.6772	1.14
7	8.5117	1.54	6.0188	1.21
8	9.2383	0.32	2.6185	0.53
9	9.4317	0.45	3.1026	0.62
10	10.1200	0.21	0.9288	0.19
11	10.6050	0.21	0.8746	0.18
12	11.4183	0.21	1.3165	0.26
13	12.2567	0.60	2.1726	0.44
14	13.3667	0.63	2.8142	0.57
Sum			**496.8561**	

DISCUSSION

Aloe vera is a medicinal plant of great value, not only in view of traditional system of medicine but also of interest in modern medical science as evident by bulk of research done till date and still on going. This is xerophytic perennial plant with fleshy leaves. Most of the activities of medicinal interest have been shown by inner succulent part of the leaves which also contains gel. Thus *Aloe vera* leaf succulent is grinded and used as it is in our study after freeze drying to retain its natural composition.

AVS was subjected to qualitative analysis for the presence of different bioactive phytochemicals. It was found to contain flavonoids, saponins, glycosides, tannins, reducing sugers, starch and sterols.

Qualitative analysis of *Aloe vera* for phytochemicals done by **S. Arunkumar and M. Muthuselvam (2009)** in aqueous, ethanolic and acetone extracts revealed presence of tannins, phlobotannins, saponins and flavonois, which is similar to our findings.(6) Another study done by **Medha Prajapati *et al.* (2011)** revealed presence of presence of same compounds along with steroids in lesser amount, which was a different finding than our results.–(7) The reason behind this may be because they have used whole dried plant powder to do the analysis. ***Karungadevi et al.* (2009)** in their study on *Aloe vera* juice and gel found presence of same compounds as our

study, except presence of steroids in *Aloe* juice.(8) The difference in results here may be because the *Aloe vera* juice they have used in study was purchased from market and might have been altered during the processing. In study *Aloe vera* gel showed negative results for the presence of steroids. According to ***Eshan and He* (2004)** the composition of *Aloe vera* differs according to the plant variety, climate, seasonal variations and age of the plant.(9) The processing method applied also affects the number and amount of active constituents ***Wang and Strong* (1995)**.(10)

AVS on HPLC analysis of methanolic extract certainly showed more phyochemical results with higher number of discrete peaks compared to the aqueous extract and that is with almost double of retention time until last peak. AVS on HPLC analysis shown 7 peaks in aqueous extract and 14 different peaks in methanolic extract. The typical resulting chromatograms of each method are shown in Figure 1. ***Azaroual et al.* (2012)** used three iferent methods for analysis of Aloe vera gel, for the UPLC method, the retention time of the final compound was 3.81 min, whilst in the case of the HPLC method with a monolithic column (HPLC/M) the time was 4.17 min, and in the case of a particulate column (HPLC/P) it was 30.7 min.(11)

Mariappan *et al.* (2012) have also done HPLC analysis of *Aloe vera* leaf and gel. They found 29 and 25 peaks for leaf and gel respectively while able to identify and measure Anthraquinone, flavonoids, alkaloids, tannins, phenols, -sitosterols etc quantitatively.(Mariappan & Shanthi, 2012) The difference in no of phytochemical with our study is may be due to different plant material and method used for analysis. HPLC analysis is known to be sensitive and machine and method specific.

CONCLUSION

Analysis reveal presence of many phytochemical compound such as Anthraquinone, flavonoids, alkaloids, tannins, phenols, β-sitosterols etc in the inner succulent gel of *Aloe vera*.

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