Quantitative Determination of Aloin in *Aloe vera* and Its Antioxidant Activity

[*1]Suman Lata Chhimwal, [2] I.P.Pandey amd [3]Shakira Malik

[1]The Himalaya Drug Company, Sahranpur Road Dehradun, UK, India
[2]Professor Emeritus, Council of Scientific & Industrial Research, Delhi-India
[3]Himalayan Institute of Pharmacy and Research, Dehradun, (Uttarakhand),India
***Email: suman15dec@yahoo.co.in**
DOI 10.51129/ujpah-2020-29-2(9)

Abstract-*Aloe vera* is a popular medicinal plant used widely by the cosmetic, pharmaceutical and food industries. The *Aloe vera* gel, which is used mostly for its positive effects on human health, contains over 75 different bioactive compounds, including aloin. A sensitive and reliable densitometric High Performance Thin Layer Chromatography method has been developed for the quantification of aloin, an anthraquinone present in *Aloe vera* leaves. Chromatographic analysis was performed using methanol extract of leaves of *Aloe vera* using solvent system ethyl acetate: Methanol: water (100:13.5:10). Detection and quantification of aloin was done by densitometric scanning at 350nm. The results of linearity range and correlation coefficient show that there was a good correlation between peak area and corresponding concentration of aloin. The method developed here in can be implemented in the analysis and routine quality control of herbal materials. Antioxidant activity of *Aloe vera* was also done by using DPPH method and found that *Aloe vera* is also a natural antioxidant.

Key words: *Aloe vera*, Aloin, HPTLC, Antioxidant activity

Introduction

The plant *Aloe vera* is universally known and widely cultivated all over India. Therapeutically, *Aloe vera* is used both internally and externally for curing various ailments. *Aloe vera* has long been used as a remedy in many cultures. As the "wonder plant" has multiple uses from being an antiseptic, anti-inflammatory agent, helps in relieving cancer and diabetes, being in cosmetic field (Davis RH, 1989). *Aloe vera* is undoubtedly, the nature's gift to humanity for cosmetic, burn and medicinal application and it remains for us to introduce it to ourselves and thank to the nature for its existence as never-ending gift. Aloe preparations including products based on both the gel and the leaf, are used among other reasons as laxatives, in creams for skin, in functional foods, and as treatment for a wide range of diseases (V. Steenkamp,2007). The gel found in the leaves is used for soothing minor burns, wounds, and various skin conditions like eczema and ringworm. The extracted Aloe juice is used internally to treat a variety of digestive condition (Kokate C K). There are more than 200 different chemical constituents found in *Aloe vera* namely amino acids, Anthraquinone, Lignin, Mono-and polysaccharides, Saponins, sterols, Vitamins, Salicylic acid, Minerals, Enzymes, in which Aloin is reported as a main chemical constituent (M. Ahmed, 2013).

Aloin is the main anthraquinone in aloe leaf, which occurs naturally as a mixture of two diastereoisomers, aloin A and aloin B. In addition to these compounds, other compounds including aloenin, aloenin B, and isoaloesin have been related to the biological properties of *Aloe vera* extracts (S. P. Joshi, 1997). *Aloe vera* also contains several potentially bioactive compounds including salicylates, magnesium, lactate, (Joshi SP, 1997). Aloe is widely used in the cosmetic, pharmaceutical and food industries because it contains antioxidants which may increase the shelf life and nutritional value of food.

HPTLC is a sophisticated and automated form of TLC. *HPTLC* is an invaluable quality assessment tools for the evaluation of active biomarker. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally numerous samples can be run in a simple analysis there by reducing analytical time with HPTLC. The analysis can be carried out using different wave lengths of light - 254nm. short wave UV light, 366nm long-wave UV light, and 302nm mid wave

light. High-performance thin layer chromatography (HPTLC) is the usual technique for the determination of individual components in *Aloe vera* leaf extracts. There are already several examples of the use of HPTLC for the analysis of aloin derivatives (Pandey, D. K., 2012) The objective of the present work was to develop an accurate, specific and reproducible method for the estimation of Aloin from *Aloe vera* and its antioxidant activity.

Material and Methods

Sample preparation

Aloe vera leaves were collected from the herbal garden of The Himalaya Drug Company, Dehradun. These were washed with water and removed the rinds. The inner gel scrapped, cut into pieces and kept dried on petridish. The dry gel particles were scratched and weighed, then dissolved in methanol (250mg in 50ml). The resulting solution was filtered. This solution was used for HPTLC analysis.

Standard solutions

Dissolved 10mg of reference compound of Alon in 10 ml of methanol. 0.1 µL of the solution contains 100mg aloin.

Chromatographic Conditions

The HPTLC system was composed of a CAMAG Linomat-5 sample applicator and CAMAG TLC scanner-3 provided with CATS software. The stationary phase was composed of pre-coated silica gel 60F254 TLC plates (20×10). Samples were administered to the plate via Linomat-5 with a 100ul Hamilton syringe. The twin through development chamber was saturated with ethyl acetate: Methanol: water (100:13.5:10) as mobile phase (Wagner and Bladt, 1996). Applied different volume of samples and standard band wise with CAMAG Linomat 5, distance from lower edge, 8mm and distance from left edge, 20mm. Evaluation was done by using CAMAG TLC Scanner and CATS evaluation software; scanning by absorbance at 350nm. Evaluation via peak area.

Antioxidant assay

The evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of (Brand-Williams et al 1995) with modifications.

The following concentrations of extracts were prepared 10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL, 50µg/mL, 60µg/mL and 70µg/mL. A stock solution of the sample (50mg/ml) was diluted for 7 concentrations. Each concentration was tested in duplicate. The portion of sample solution (100µl) was mixed with 300µl of 0.2mM 1, 1-Diphenyl-2-2picrylhydrazyl (DPPH, in 96% distilled ethanol) and allowed to stand at room temperature for 30 minute under light protection. The absorbance was measured at 517nm.The scavenging activity of the samples at corresponding intensity of quenching DPPH. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The difference in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Inhibition % = Ac-As/Ac×100

Where Ac is the absorbance of the control As is the absorbance of the sample

Results and Discussion

The Rf values and color of the resolved bands were noted after development of the silica plate upto the height of 8.5cm. The bands subjecting to aloin were found to be yellow in color under 366nm. The Rf was found to be 0.36. The densitometric scanning at 350nm of aloin and A*loe vera* are in fig-1 and fig-2 respectively. The spectra generated for aloin and methanol extract of *Aloe vera* leaves at wavelength range of 200-400 nm also matches as given in Fig-3, which indicates the presence of aloin in the sample.

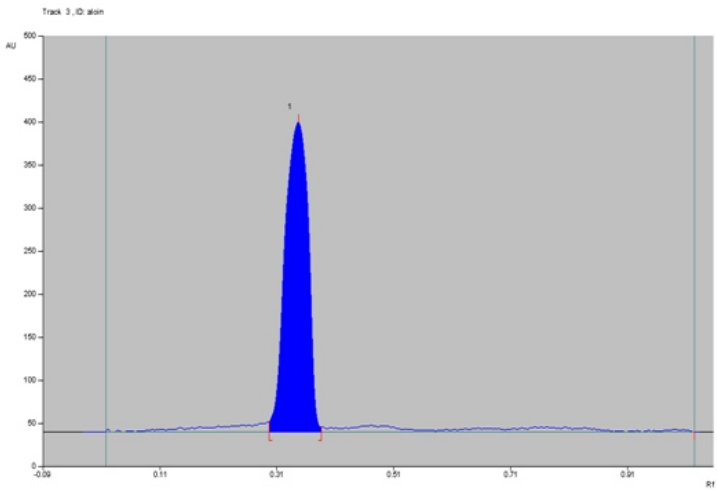

Figure-1 HPTLC Chromatogram of standerd aloin at Rf 0.36 at 350nm

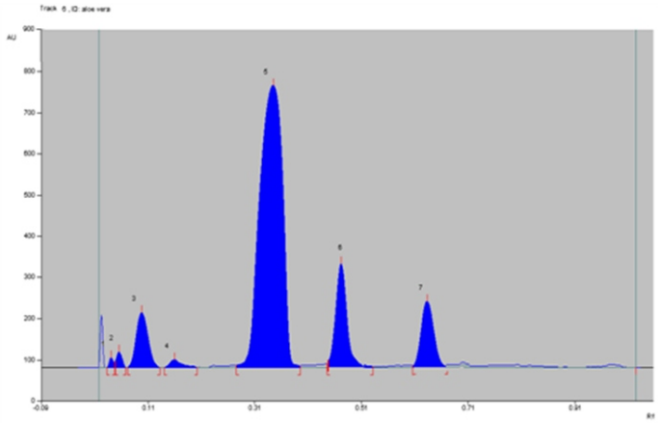

Figure-2 HPTLC Chromatogram of aloe vera at 350nm

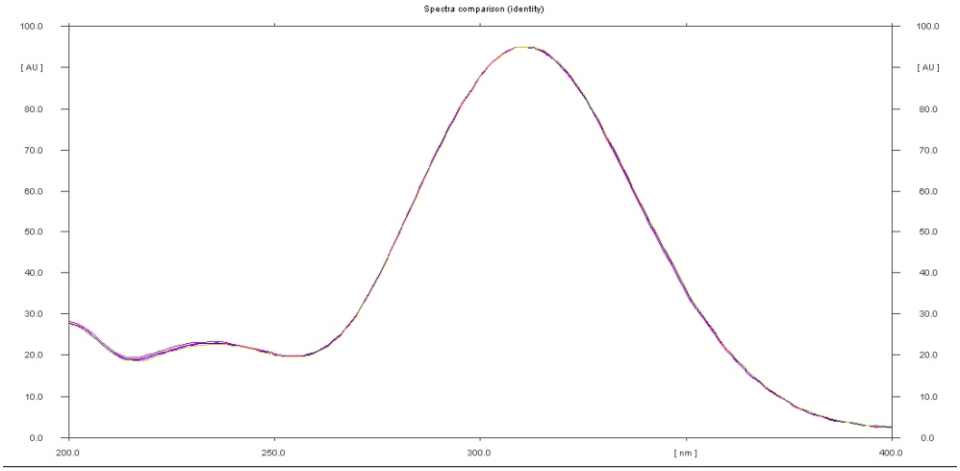

Figure-3 Overlaying of absorption spectra of methanol extract of *Aloe vera* with standard aloin.

The selected solvent system gave well defined peak resolution. With the help of the peaks and analyzing the UV spectra, the band at Rf 0.36 was idenfified as aloin. The TLC plates were visualized at 254nm and 366nm in camag visulizing cabinet. Better visulization was obtained at 366nm because of the florescence of aloin. Linear calibration curve was found between 200ng-1000ng of aloin. The correlation coefficient for a calibration curve was founf to be 0.99957 for aloin.

The regression equation for the calibration plot for aloin is Y=283.238+2.183X. percentage of aloin was determined by using the peak area parameter. This HPTLC method is specific for aloin because it resolved the compound (Rf=0.36) well in the presence of other components in *Aloe vera*. The aloin content was found 2.43%w/w. The method allows reliable quantification of aloin and good resolution from other constituents of *Aloe vera*.

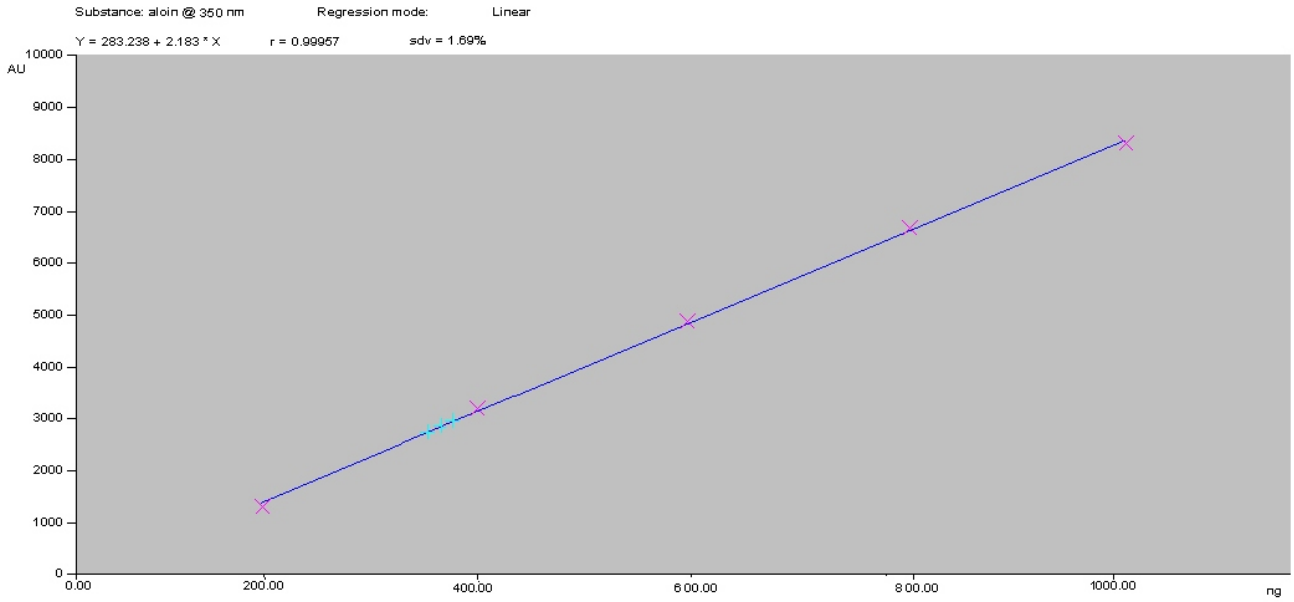

Figure-4 Linear calibration curve of aloin (200ng-1000ng)

Antioxidant activity of *Aloe vera* leaves

Our study emphasizes the antioxidant potential of *Aloe vera* methanolic leaf extracts. Methanolic extract induced the best extraction yield and more complex composition of phenolics (Pop RM 2013). The methanol extracts of *A.vera* were also screened previously for their in vitro antioxidant activity (Lopez A 2013). Biological activities of *A. vera* may be due to the synergistic action of these compounds, rather than from a single defined component [46].

The antioxidant activity of methanolic extract of A*loe vera* leaves is determined by the free radical DPPH reduction method, Fig-5. DPPH radical scavenging method is an extensive procedure to evaluate the free radical scavenging ability of various samples (Brand-williams W,1995). The effect of antioxidants on DPPH radical scavenging was supposed to be due to their hydrogen donating ability.

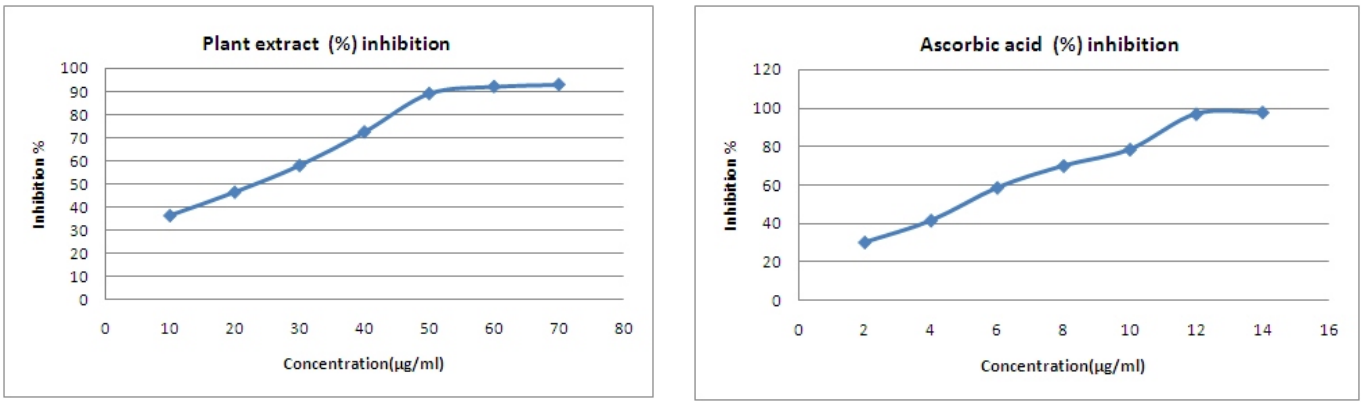

Figure-5 DPPH radical scavenging activity of aleo vera plant extract and ascorbic acid

The antioxidant activity has been given from the IC_{50}. It is the concentration of the antioxidant giving 50% inhibition of DPPH in the test solution. The evaluation of the antioxidant activity of the methanolic extract of *aloe vera* and ascorbic acid show that the IC_{50} values are 27.92µg/mL and 5.19µg/mL respectively. Scanning of *aloe vera* plate at 350nm shows the presence of more than seven constituents present in it as shown in Fig-2. The antioxidant activity of *A. vera* may be due to the synergistic action of these compounds, or may be due to the active constituent aloin.As reported earliar the aloin is known as antioxidant, antimicrobial, anticancer, anti-inflammatory, (S.kumar 2017).

Conclusion

Among the Complex mixtures of biologically active compounds in the leaves of *Aloe vera*, aloin can be used as an analytical marker to determine the quality of plant material. The aloin content was found 2.43%w/w. The active ingredients hidden in its leaves have the power to soothe human life and health in many ways. The present study showed that *A. vera* is a promising source of bioactive compounds which has a good antioxidant activity. The in vitro antioxidant activity showed that the *Aloe vera* has a good antioxidant activity. Good antioxidant properties of the *Aloe vera* could be considered for applications in food, medicine and cosmetic industries.

References

1. Ahmed, M and Hussain, F. Chemical composition and biochemical activity of *Aloe vera (Aloe barbadensi*s Miller) leaves *IJCBS.,* 2013, (3): 29-33.

2. Brand-williams, W.; Cuvelier, M.E. and Berset C. Use of free radical method to evaluate antioxidant activity. *Food Sci. Technol.,* 1995,28(1): 25-30.

3. Davis, R.H. *Aloe vera*: A Scientific Approach. New York: Vantage Press; 1989.1997

4. Joshi, S.P. "Chemical Constituents and Biological Activity of *Aloe barbadensis* A Review," *Journal of Medicinal and Aromatic Plant Science,* 1997,(20):768-773.

5. Kokate, C.K. and Purohit, A.D. Pharmacognosy Edition14, Nirali Prakashan, Pune, Chap, 8:23-30.

6. Lopez, A.; de, Tangil, M.S.; Orellana, O.V.; Ramirez, A.S. and Rico M. Phenolic constituents, antioxidant and preliminary anti mycoplasmic activities of leaf skin and flowers of *Aloe vera* (L.) Burm. f. (syn. *A. barbadensis* Mill.) *Molecules*. 2013,(18):494-254.

7. Pandey, D.K.; Tabarak, Malik and Banik, R.M. Quantitative estimation of barbaloin in *Aloe vera* and its commercial formulation by using HPTLC. *Int J Med Aroma Plants,* 2012:420-427.

8. Pop, R.M.; Csernatoni, F; Ranga, F; Fetea, F and Socaciu, C. HPLC-UV Analysis coupled with chemometry to identify phenolic biomarkers from medicinalplants, used as ingredients in two food supplement formulas.Food Sci Technol. 2013,70(2):99-107.

9. S. Kumar, A; Yadav, M; Yadav, J.P. and Yadav. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of *Aloe vera* (L.) BMC Res Notes. 2017, 10(1):1-12

10. V. Steenkamp and M. J. Stewart, "Medicinal applications and toxicological activities of Aloe products," *Pharmaceutical Biology*, 2007,45,(5): 411-420.

11. Wagner, H and Bladt, S. Plant drug analysis. A thin layer chromatography atlas, 1996, 62-64.