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ISOLATION OF ALOIN FROM *ALOE VERA,* ITS CHARACTERIZATION AND EVALUATION FOR ANTIOXIDANT ACTIVITY

Patidar Arun[1*],
Bhayadiya R.K.[1], Nimita Manocha[1], Pathan J.K. [1], Dubey P.K. [1]

[1]Department of Pharmaceutical Chemistry, Swami Vivekanand College of Pharmacy, Indore– 452020, India

ABSTRACT

Aloin an active compound obtained from the Aloe Vera species is already confirmed to exhibit laxative, anti-inflammatory and anticancer effect. However, scientifically proven information available are limited for the in-vivo supplements of Aloin based drugs.

The present protocol describes a simple and efficient method for isolation of Aloin from Aloe Vera. Column chromatography was used to isolate Aloin from dried crude extract of Aloe Vera. The ethanolic extract of Aloe Vera leaf skin was fractionated by column chromatography using isocratic biphasic solvent system ethyl acetate-methanol-water (77:13:10) as mobile phase. Silica gel G (60-200 mesh) used as stationary phase. This eluent system gives optimal separation of leaf components. The eluents were characterized in way of appearance, TLC, melting point, solubility, UV spectroscopy and IR spectroscopy. The Aloin was obtained as yellow crystalline solid. Antioxidant activity was carried out using DPPH radical-scavenging method at Different concentrations (100 and 250µg/ml) using ascorbic acid as a standard. Aloin exhibited potent antioxidant activity (68% at 100 µg/ml and 73% at 250µg/ml) as compared to ascorbic acid.

Key words: *Aloin, Antioxidant, DPPH.*

Correspondence to Author

Patidar Arun

Department of Pharmaceutical Chemistry, Swami Vivekanand College of Pharmacy, Indore– 452020, India

Email: arunpatidar21@gmail.com

INTRODUCTION

Many diseases are caused by oxidative stress. Accelerated cell oxidation contributes to cardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease, and even a decline in energy and endurance. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. Recently, natural plants have received much attention as sources of biological active substances including antioxidants.

Aloe Vera is a stemless or very short-stemmed succulent plant growing to 60–100 cm

(24–39 in) tall, spreading by offsets. The bitter aloes consist of free anthraquinones and their derivatives, Barbaloin, aloe-emodin-9-anthrone, Isobarbaloin, Anthrone-C-glycosides and chromones.[6] In large amounts these compounds exert a powerful purgative effect, but when smaller they appear to aid absorption from the gut, are potent antimicrobial agents and possess powerful analgesic effects[3]. They also reduce the formation of melanin and any tendency to hyper-pigmentation. Lignin with their penetrative ability to carry other active ingredients deep into the skin to nourish the dermis.[5]

Aloin an active compound obtained from the *Aloe Vera* species is already confirmed to exhibit an anti-inflammatory[2], anticancer[3] effect. The most well-documented yet mechanistically unknown activity of aloin is its purgative/laxative action in animals[6]. **Aloin**, also known as Barbaloin is a bitter, yellow-brown colored compound noted in the exudate of at least 68 *Aloe* species at levels from 0.1 to 6.6% of leaf dry weight (making between 3% and 35% of the toal exudates) , and in another 17 species at indeterminate levels. It is used as a stimulant-laxative, treating constipation by inducing bowel movements. However, scientifically proven information available are limited for the *in-vivo* supplements of Aloin based drugs.

MATERIAL AND METHOD:

1. Collection of Aloe Vera:

Fresh Aloe Vera was collected from the medicinal garden of Swami Vivekanand College of Pharmacy, Indore.

2.Extraction of Aloin:

Aloe Vera leaves were cut from plants and clean the leaves by using cotton. Then cuts were mad on leaves and kept overnight to remove gel from leaves. Then gel dissolved in ethanol and filtered it to remove impurities, evaporated methanol to concentrate the extract.[1]

3.TLC *Aloe Vera* extract:

TLC was performed on TLC plates using silica gel G (for TLC) as a stationary phase and various solvents are used as mobile phase to find out the better solvent system for maximum separation of various compounds of *Aloe Vera*. Finally ethyl acetate (EtOAc), methanol (MeOH) and water (H_2O) in ratio of 77:13:10 (V/V/V) was fined out as a mobile phase for optimum separation, and R_f value of Aloin was found to be 0.47. Aloin also gives yellow florescence in UV light.[10]

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

TLC Size : 75 mm
Stationary Phase : silica gel G
Mobile phase : ethyl acetate : methanol : water (77:13:10 ,V/V/V)
Detection : Iodine chamber

4. Isolation of Aloin by Column chromatography:

Ethanolic extract was subjected on column chromatography (480×18 mm**)** using silica gel G (60-200 mesh) as stationary phase and ethyl acetate, methanol and water (77:13:10) as mobile phase. This eluent system gives optimal separation of leaf components. Total 32 fractions were collected, TLC was performed simultaneously and fractions were mixed together which has same R_f value. Than different fractions were air dried. However, one major problem with this system was that water was present in each fraction that was removed by freeze-drying.[10]

4.1 Column preparation:

Silica gel G 100-200 mesh was activated in oven and mobile phase ethyl acetate, methanol and water (77:13:10,V/V/V) was prepared. Activated silica was mix with mobile phase than poured in column, after that bad of silica packed with cotton.

4.2 Sample preparation and loading:

Extract was mixed with a small portion of silica than poured in column, trapped it to settle down the mixture of silica and extract. After sample loading sample was packed with cotton which prevents the disturbance of sample bad when solvent was poured.

4.3 Solvent running and fractions collection:

After sample loading the mobile phase of ethyl acetate, methanol and water

(77:13:10,V/V/V) was poured in column and allowed to run from the column. The elution was collected and TLC was performed simultaneously, on the basis of TLC which elution gave similar R_f value were pulled together and dried.

4.4 TLC:

TLC plates were prepared using silica gel G (for TLC). A slurry of silica gel was prepared and apply on TLC plates than dried in oven at 110^0C for 1 hour. TLC was performed using silica gel G (for TLC) as a stationary phase and ethyl acetate, methanol and water (77:13:10,V/V/V) as mobile phase.

In-vitro antioxidant Study (Free Radical Scavenging Activity):

The free radical scavenging activity of the aloin was measured by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) using the method described by Mohammad Ali et al. (2010). Different concentrations (100 and 250µg/ml) of aloin were added, at an equal volume to methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minute then the absorbance was measured at 517nm by using spectrophotometer. Reference standard compound being used was Vitamin C 100 and 250µg/ml.[7]

The percent DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) =100[(A_0-A_1)/A_0]

Where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in presence of the standard sample or synthesized compound[44].

RESULTS AND DISCUSSION:

Table 1 : Characterization of different fractions of Column Chromatography:

Fraction No.	Appearance	R_f Value	UV Range (nm)	Melting Point (^{0}C)
1	Brownish-yellow	0.76	228,246,252,284	138-142
2	yellow	0.47	260,270,296,358	142-146
3	Light-yellow	0.38	274,300	140-142
4	brownish-yellow	0.19	240,252,298	132-134

Mobile phase *:-* ethyl acetate : methanol : water (77:13:10)

Ultraviolet-Visible Spectrum of Aloin:

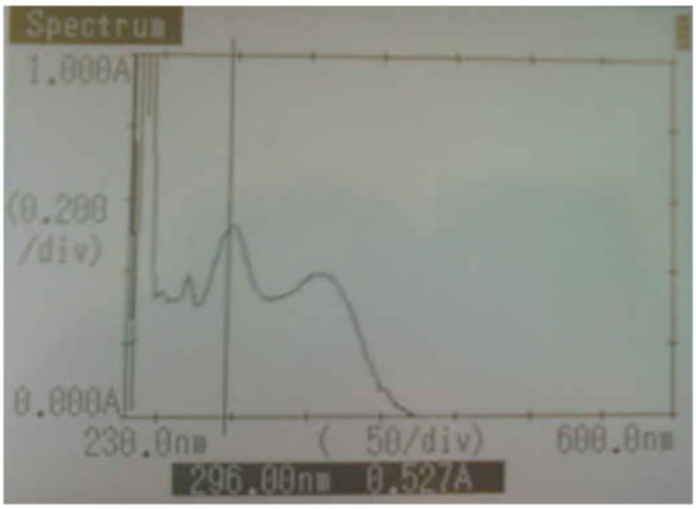
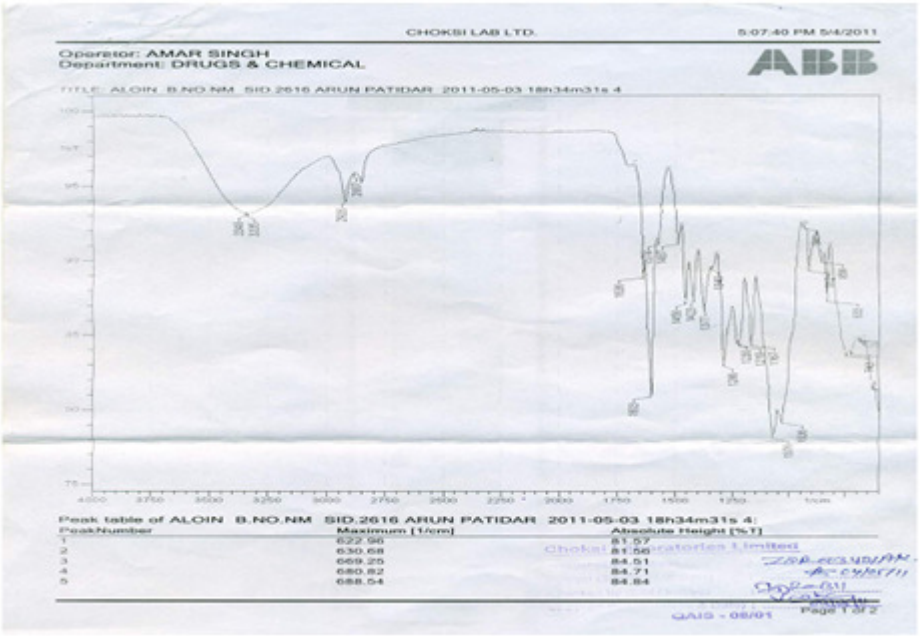


Fig 8: UV Visible Spectrum of Aloin

UV λmax (nm): 260,270,296,358

Infra Red Spectral Study of Aloin:



Spectral data of Aloin;

1H-NMR (CH3OH-d4) ä ppm: 5 (s, 2, phenolic), 6.58 (s, 1, aromatic), 6.71 (s, 1, aromatic), 6.78 (s, 1, aromatic), 7.23 (s, 1, aromatic), 6.65 (s, 1, 11 aromatic), 4.37 (s, 2, H on 10-C), 4.79 (s, 2, CH2 of primary OH), 2.0 (s, 1, primary OH), 4.44, 3.4, 3.49, 3.76, 3.79, 3.54, 2.0 (glucose unit); (M+) 418.6; IR (KBr) 3348 cm^{-1} (O-H str), 1602 cm^{-1} (C=C str), 2925 cm^{-1} (C-H str), 1635 cm^{-1} (C=O str), 1456 cm^{-1} (C-H str)

In-vitro antioxidant Study (Free Radical Scavenging Activity):

Table 2: In-vitro antioxidant activity study of Aloin

Compounds	(Absorbance / % Scavenging)	
	100µg/ml	250 µg/ml
Control	0.302/00	0.302/00
Ascorbic acid	0.056/81	0.060/80
Aloin	0.078/68	0.92/73

DISCUSSION:

Aloin was successfully isolated from *Aloe Vera* by column chromatography using isocratic elution with ethyl acetate : methanol : water (77:13:10 ,V/V/V) as mobile phase.

The physico-chemical properties of the isolated compound comply with that of pure aloin as reported in reference data. The compound was brownish yellow crystals, melting point 146-148^{0}C, R_f 0.47 with mobile phase - ethyl acetate : methanol : water (77:13:10 ,V/V/V) . It gave UV λmax of 260,270,296,358 nm. Spectral data of IR, ^{1}HNMR and Mass analysis of Aloin showed confirmation of its structure, the spectrum data of isolated compound was similar with that of aloin as given in reference data.

Antioxidant activity was carried out using DPPH radical-scavenging method at Different concentrations (100 and 250µg/ml) using ascorbic acid as a standard. Aloin exhibited potent antioxidant activity (68% at 100 µg/ml) as compared to ascorbic acid. It also exhibited good antioxidant activity with percentage scavenging 73% at 250 µg/ml.

CONCLUSION:

Aloin was successfully isolated from *Aloe Vera.* The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. It was found that the radical- scavenging activities of

all the extracts increased with increasing concentration.

Therefore, this research has opened new doors for possible antioxidant activity of natural available lead molecules. Thus, it is worthwhile to explore the applications of aloin in drug discovery. Research is in progressing to isolate aloin and identify the antioxidant activity of aloin.

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