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QUALITATIVE AND QUANTITATIVE PROFILE OF ALOIN ISOLATED FROM *ALOE VERA*

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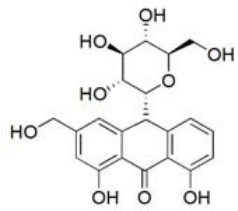
ABSTRACT
A.vera is known to contain well over 100 separate ingredients or constituents between those found in the leaf and those found in the mucilaginous gel inside the leaf. It is also known that some of the ingredients found in the leaf such as Aloin, or the Emodins are recognized as having laxative and antimicrobial properties. Aloin is Folk Traditional Medicine used for Healing of minor cutaneous injuries, such a blisters, abrasions, cuts, burns, bites and scares, protection and care of external skin, such as cleansing, moisturizing, tightening and in mixes as sunscreens and anti-chapping compounds. In the present work, we have investigated the qualitative and quantitative determination of Aloin isolated from the *A.vera*. Qualitative estimation was carried out by treating the sample with bromine water and thin layer chromatographic (TLC) method. The simultaneous determination of the Aloin was carried out by HPLC technique. HPLC separation was performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5µ) using methanol (A) and 0.34% acetic acid (B) using a isocratic elution as follow: 0–30 min, 40%A–80% A, 60%B–20% B. The flow rate was 1.0 mL/min, and a column temperature of 25°C. The injection volume was 25µl, and UV detection was effected at 297.5 nm.
Keywords: Aloin, *A.vera,* HPLC, TLC & isolation.

INTRODUCTION

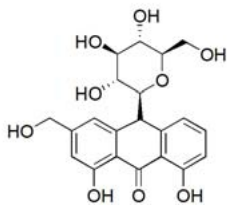
Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants to be potential sources of medicinal substances[1]. For centuries, plants and plants products have been used for treating various illnesses. Today, several medicinal plants and their products are still in use, being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market[2]. *Aleo vera* belonging to the family Liliaceae has been referred to as the "First Aid Plant". The word 'aloe' has its roots in the Arabic word 'alloeh', which means 'radiance'. With the recent resurgence of herbal products as a part of 'green movement', aloe vera is witnessing a new renaissance across the world[3]. The Aloe plant grows in different sizes. It can be 10 inches to 30 inches. It grows in a rosette formation. This shape helps the Aloe plant catch as much rain as possible. It also helps shade itself from the tremendous heat of the desert. The *Aloe vera* varies in color from light to dark green. It usually is darker than regular cactus. The *Aloe vera* has long flat leaves. The edges have points but they are not sharp. It has a waxy coating to keep the moisture inside. The species in *Aloe vera* L. have been used as ethnic medicines in many different countries for centuries, possessing functions, such as anti-cancer, anti-inflammatory, anti-virus, evacuating, protecting liver, and increasing immunity (Lu *et al*, 2008). *Aloe vera* L. has been most commonly used as medicine, healthy foods, and cosmetics nowadays[4]. The main chemical constituents are hydroxyanthraquinone derivatives (25-40%) viz., aloin (=barbaloin, a mixture of aloin A & B, the diastereoisomeric 10-C glucosides of aloe-emodin anthrone) and 7-hydroxyaloin isomers, aloe emodin, chrysophanol; derivatives viz., aloeresin B (=aloesin, upto 30%) with its p-coumaryl derivative aloeresins A & C, and the aglycone aloesone[5].The common names are Aloe, aloe capensis, aloe spicata, aloe vera, Barbados aloe, Cape aloe, chirukattali (India), Curacao aloe, Ghai kunwar (India), Ghikumar (India), Indian aloes, kumari (Sanskrit), laloi (Haiti), lohoi (Vietnam), luhui (Chinese), nohwa (Korean), rokai (Japanese), sabilla (Cuba), Socotrine aloe, subr (Arabic), Zanzibar aloe[6].

Fig.1 ***Aloe vera***

The Anthraquinones/anthrones present in are Aloe-emodin, aloetic-acid, anthranol, aloin A and B (or collectively known as barbaloin), isobarbaloin, emodin, ester of cinnamic acid. Aloin, (10-glucopyranosyl-1,8-dihydroxy-3-(hydroxylmethyl)-9,(10H)-anthracenone, a bio active molecule in *Aloe vera* is already known to have antimicrobial effect[7].



aloin A



aloin B

Plant Material

The Leaves of *A.vera* were obtained from the medicinal garden of L.N.C.P. Bhopal (M.P.) in the month of March 2011.The plant was authenticated and a voucher specimen was deposited.

Isolation of Aloin

Fresh leaves were harvested, washed and epidermis was removed to get mucilaginous and colourless parenchyma. It was homogenized in a high-speed homogenizer and diluted with distilled water to obtain a thin solution. Now the solution was filtered through filter press to obtain a clear liquid. The liquid was concentrated to 5% under reduced pressure. For converting gel to powder, it was spray dried after bringing total dissolved solids to about 15% by concentration under reduced pressure[8].

Qualitative analysis by Thin Layer Chromatography

For thin layer chromatographic studies of Aloin, precoated Silca gel F_{254} aluminum plates (20 X 20cm) were used[12].The Aloin was

separated using ethyl acetate: methanol: water [100:13.5:10]. The colour and R_f values were recorded by sprayed with 5% ethanolic potassium hydroxide.

Quantitative estimation of Aloin by HPLC

The HPLC analysis was performed using a LC-100, Cyberlab™, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations.The mobile phase consisted of methanol (A) and 0.34% acetic acid (B) using a isocratic elution as follow: 0–30 min, 40%A–80% A, 60%B–20% B. The flow rate was 1.0 mL/min, and a column temperature of 25°C. The injection volume was 25µl, and UV detection was effected at 297.5 nm. HPLC grade solvents were obtained from Shyam brothers, 27-sindhi market, Bhopal. After isolation the aloin (10µg/ml) were subjected to HPLC column and the obtained records were superimposed on the retention time values of the standard aloin. Besides the collected data, compound I had the same retention time in HPLC with the reference substance of aloin A, so compound I was identified as aloin A. According to the similar data with aloin A except the retention time, compound II was identified as the isomer of Aloin A, named Aloin B.

Result and Discussion

Aloe vera leaves which have been reported to have medicinal uses. Keeping in view of the ethno-pharmacological importance of leaves *A.vera* the isolation of important phytoconstituent Aloin was undertaken for standardization. Organoleptic evaluation (Table1) showed the following characters: colour- light brown with bitter taste. The solubility study of sample was determined (Table 1). The isolated sample was subjected for qualitative estimation by treated with bromine water (Table 1) and TLC analysis (Table 2). The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines. HPLC separations of isolated samples with reference to standard were performed on a Cyber Lab C-18 column (250 x 4.0 mm, 5µ). Thus chromatographic fingerprint should be considered to evaluate the quality of herbal medicines globally considering multiple constituents present in the herbal medicines[9].

CONCLUSION

The Aloin was isolated from *A.vera* and identified by TLC and HPLC methods. Aloin is therapeutically used as a laxative. In-vivo research trials show anti-inflammatory, anti-microbial and diet supplementary. It is Bio-active compound which causes cellular modulation and immunological alterations. Hence, this plant and their phytoconstituents are useful for the above disease affecting the mankind. Further studies required to proof the potential of this plant.

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Table 1: Physiochemical Evaluation of Aloin

S.No.	Characteristics	Observation	Test method
1.	Identification	[+]ve precipitated	Presence of mucilage & bromine water
2.	Colour	Brown colour powder	Visual
3.	Taste	Bitter	Organoleptic
4.	Solubility in water	(+)ve (90%)	visual
5.	Solubility in ethyl alcohol	(+)ve(70%)	visual
6.	Melting point	148(Aloin A) 139(Aloin B)	I.P.1996

[+]ve = Present

Table 2 : TLC Profile of Aloin

S. No.	Solvent System	R_f of Sample	R_f of Sample	Inference
1.	Ethylacetate:methanol:water [100:13.5:10]	0.24 0.46 0.7	0.25 0.48 ------	Aloinoside A& B Aloin unknown

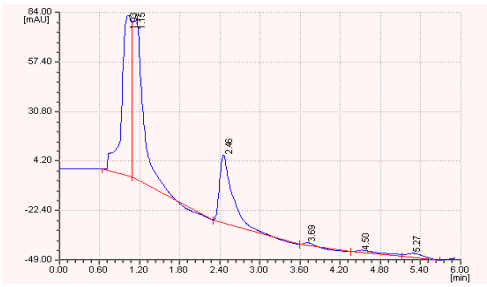

Fig:2 HPLC chromatogram of Sample

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