



EXTRACTION, IDENTIFICATION AND ANTIBACTERIAL ACTIVITY OF ALOIN EXTRACTED FROM ALOE VERA

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Article Received on 20/06/2017

Article Revised on 10/07/2017

Article Accepted on 31/07/2017

ABSTRACT

Aloe Vera *Barbadensis miller* is a vital plant consist of various phytochemicals such as aloin (anthraquinones), vitamins, enzymes, minerals, polysaccharides, saponins, lignins, amino acids, fatty acids & salicylic acid having significant therapeutic effects. The present work is to examine antimicrobial activity of aloin extracted from dried Aloe Vera leaves powder against gram negative bacteria *E. coli* & gram positive bacteria *Bacillus subtilis* respectively by using agar dilution test & it was observed that it impart an adequate antimicrobial activity against the tested micro-organisms.

KEYWORDS: Aloin, Aloe Vera, extraction, antibacterial property.

INTRODUCTION

Aloe barbedensis miller is a vital medicinal plant that belongs to *Liliaceae* family & genus Aloe. Aloe is a perennial succulent xerophytic cactus like plant with fleshy & thick grayish green leaves growing to about 50-70cm with serrated edges & little spines^[1] grows in hot dry climate in the world & cultivated for agricultural & medicinal uses. Aloe Vera has a wide range of applications from topical applications to in vivo

applications^[2] like intestinal disorders, liver disease etc. The vital parts of aloe are yellow latex & clear gel which is obtained from Parenchymatic cells of leaf. Aloe Vera gel is made up of 75 different components even though 95-99% of it is water.^[3] The different bioactive components have different pharmacological activities. The phytoconstituents present in Aloe Vera are as follows:

VITAMINS	Vitamin A,C,E,D & B12
ENZYMES	Lipases, proteases, kinases
MINERALS	Ca,Na,K,Mg,Mn,Cu,Zn,Cr,Se
SUGARS	Mono & Poly Saccharides, glucomannans
ANTHRAQUINONES	Most important: Aloin & Emodin
LIGNINS	Inert except when in topical treatments
SAPONINS	3% of Aloe Vera gel
FATTY ACIDS	Cholesterol, Campersterol, sisosterol&lupeol
SALICYCLIC ACID	Aspirin like
AMINO ACIDS	20 Amino Acids

The main constituents of latex are anthraquinones including hydroxyanthracene derivatives Aloin A, Aloin B, Aloe Emodin. Aloin is a most important constituent of Aloe Vera which is also known as Barbaloin. Aloin is yellow color compound having molecular formula $C_{21}H_{22}O_9$ & Molecular weight 418. Its IUPAC name is 8-Dihydroxy-10-D-glucopyranosyl)-3-hydroxymethyl)-9(10H)-anthracenone.^[4] Aloin is a mixture of two diastereoisomers i.e. Aloin A & Aloin B which are known as Barbaloin & Isobarbaloin respectively. Aloin is an anthraquinone glycoside which is localized in outer rind

of aloe plant. Studies of aloin biosynthesis indicates that Aloin B is preferentially formed.^[5] In vivo studies have shown that orally administered Aloin is poorly absorbed by GI tract & metabolized by intestinal micro flora to Aloe emodin which is oxidized form of Aloin, which is readily absorbed.^[6] Aloin is used as purgative agent to maintain digestion system by inducing peristaltic contractions.^[7] Aloin is a major bioactive constitute which is used in numerous medications such as anticancer, antiviral, ant diabetic, antiviral, anti-inflammatory, antioxidant, stimulates the immune

system^[8], bittering agent in alcohol beverages & as laxative agents.

The present studies include extraction of Aloin from Aloe Vera using soxhlet apparatus method, characterization & Analysis of Aloin by using UV spectrophotometer, Thin Layer Chromatography (TLC), & IR Spectroscopy. Examine Antibacterial Activity against gram positive bacteria & gram negative bacteria strains respectively.

EXPERIMENTAL: Extraction of Aloin from Aloe Vera.

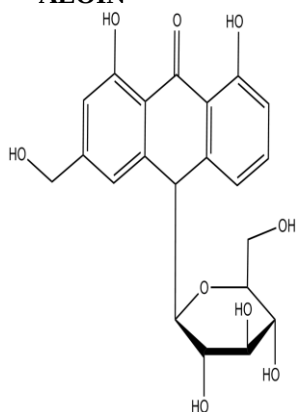
Method: Aloin was extracted from Aloe Vera by Soxhlet extraction method.

Materials required: Aloe Vera leaves, methanol.

SOXHLET EXTRACTION

Aloe Vera leaves were collected, washed & rinds were removed, inner gel were scrapped out & cut into pieces & solar dried for 10 days at 30-45°C & dry gel particles were collected. It was observed that soxhlet method is one of the best method for recovering Aloin as it gave maximum yield. Powdered the dry gel particles & 2.5g of Aloe Vera powder was taken in soxhlet with 100ml methanol. The extraction was carried out for 20-24hrs. Sample was collected at the end which was free from dry gel & was stored in a freezer & analyzed.^[9]

1. EVALUATION & CHARACTERIZATION OF ALOIN



ALOIN STRUCTURE



ISOLATED ALOIN

$\lambda_{\max}$	Absorbance
279	1.179

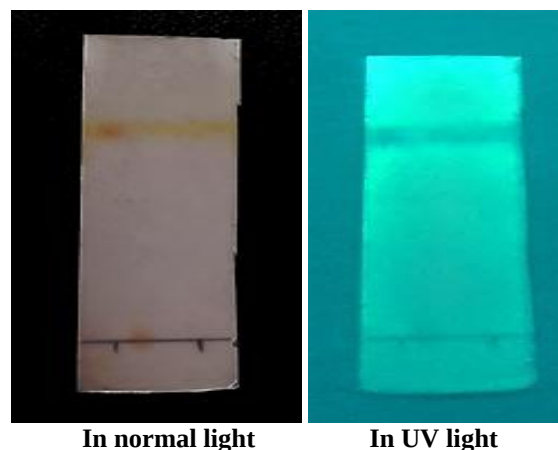
PHYSICAL AND CHEMICAL PROPERTIES OF ALOIN

- MOLECULAR FORMULA : $C_{21}H_{22}O_9$.
- MOLECULAR WEIGHT : 418.398 g/mol.
- MELTING POINT : 148°C.
- POWDER FORM : Pale yellow to greenish yellow color.
- ODOUR : Slight odour of Aloe to odourless.
- SOLUBILITY : Methanol, sparingly soluble in water, ethanol, acetone.
- SENSITIVITY : High temperature.

2. IDENTIFICATION OF ALOIN

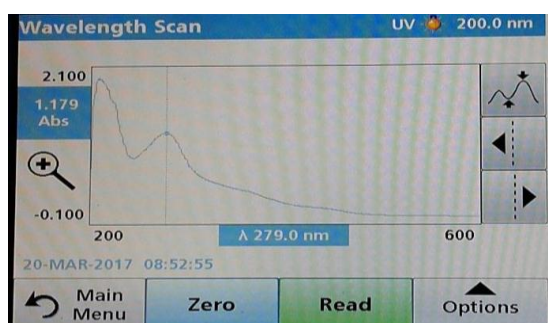
a) THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS

- SAMPLE DETAILS : Aloin.
- ADSORBENT : Percoated silica gel.
- SOLVENT SYSTEM : Ethylacetate: methanol: water(10:1.7:1.3).
- SAMPLE PREPARATION: Isolated Aloin(liq) was applied on TLC plate with the aid of capillary tube.
- DETECTION : Recorded by spraying with 5% ethanolic KOH (Rf value = 0.47).^[10]

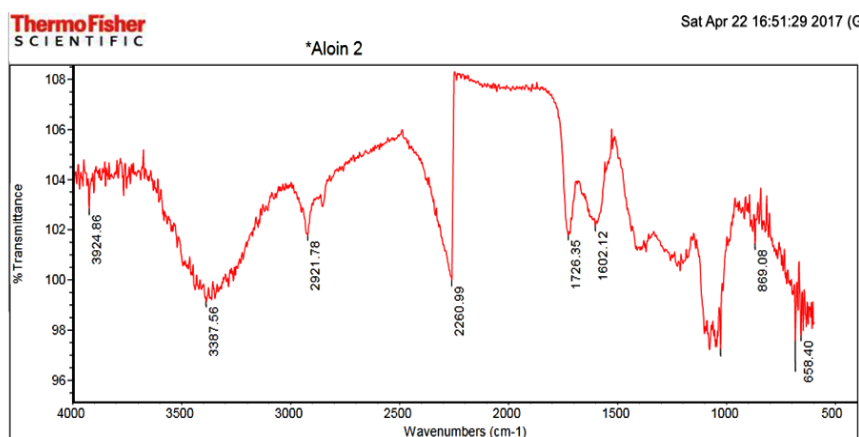


b) ULTRA VIOLET-SPECTROPHOTOMETER ANALYSIS

The UV range of sample was lie within the range 250 to 370 nm^[10] & isolated compound Aloin is lies within the range i.e. 279nm along with the absorbance 1.179. One drop of isolated Aloin were mixed with 95% methanol and $\lambda_{\max}$ were determined.



c) IDENTIFICATION BY IR SPECTROSCOPY



Frequency (cm ⁻¹)	Functional group
3387.56	OH aliphatic
2260.99	OH group
2921.78	CH stretch in Alkane
1726.35	CO(carbonyl stretching)
1602	C=C bending
3924.86	shows unsaturation
869.08	CH Alkene bending
658.40	O-H Phenol
1602.12	C=C bending
1100	Ether (medium)

ANTIBACTERIAL ACTIVITY

Antibacterial activity of Aloin was studied against *E. coli* & *Bacillus subtilis*.

PROCEDURE

The in Vitro antibacterial activity of isolated Aloin was carried out by Agar dilution method.

i) PREPARATION OF LB AGAR & LB BROTH

4gm of LB Agar was measured and added to the 100ml distilled water in a conical flask & 2.5gm of LB Broth were taken and added to the 100ml of distilled water then both are autoclaved, LB Agar & LB Broth was autoclaved for 3hrs then LB Agar was used for plating of autoclaved Petri plates where Microorganism was added to the LB Broth in a shaking chamber at 37° Celsius for 24hrs for microorganism growth.

ii) DETERMINATION OF ANTIBACTERIAL ACTIVITY

Agar coated Petri plates were coated by 100microlitres of LB Broth containing gram positive *Bacillus subtilis* & gram negative *E.Coli* respectively for determination of culture growth. Then plates having coating of LB broth containing microorganism was loaded by 10microlitre of isolated product Aloin to determine the antibacterial activity of isolated natural product i.e. Aloin.

iii) ANTIBACTERIAL ACTIVITY SHOWN BY ALOIN AGAINST *E.COLI* & *Bacillus Subtilis* AS FOLLOWS

Fig1: Plate of gram negative bacteria *E. coli*.



Fig 2: Plate of gram positive bacteria *Bacillus subtilis*.



Fig 3: Zone of inhibition by 10µl of Aloin loaded on *E. coli* culture plate.



Fig 4: Zone of inhibition by 10µl of Aloin loaded on *Bacillus subtilis* culture plate.

DISCUSSION AND CONCLUSION

Aloin was extracted out from Aloe Vera leaves using soxhelt extraction method which is observed a best method for maximum recovery of Aloin. Isolated Aloin is further identified and characterized by UV visible spectrophotometer, IR spectroscopy & TLC. Aloin have shown antibacterial activity against gram positive bacteria (*Bacillus Subtilis*) & gram negative bacteria (*E.coli*) respectively.

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