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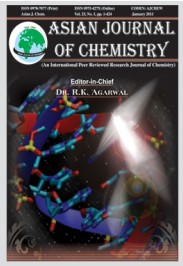

Extraction and Preliminary Analysis of Aloin Obtained from *Aloe barbadensis* Miller

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Extraction of aloin from the plant *Aloe barbadensis* Miller has been accomplished by a sequence of steps. Preliminary investigations like TLC, UV-Vis assay, nitrogen content, moisture content, ash content were carried out. The results are tabulated and a comparison of the results obtained with soil grown plant and *in vitro* cultured plant were also presented.

Key Words: Aloin, TLC, UV-Vis assay, Kjeldhal method, *In vitro* cultured plant.

INTRODUCTION

Aloin is a lemon yellow coloured crystalline compound isolated from the aloe plant and useful for treating the constipation[1]. Electrolyte imbalance, diarrhea and abdominal pain are the common side-effects of the drug if used in higher doses for prolonged time[2]. As aloin can cause uterine contraction[3], pregnant women should avoid ingesting aloe products. It is an anthraquinone glycoside[4]. Aloin is usually extracted from aloe latex, the bitter yellow exudate that seeps out from just underneath the skin of aloe leaves. The latex, also called aloe juice is then dried and powdered to make the final products for medicinal use[5].

EXPERIMENTAL

All the raw materials used are procured from Biomax Life Scienes and the chemicals are purchased from the market. The following is the procedure followed to isolate aloin.

Aloe juice extracted from the plant → Spray drying of aloe → Extraction in methanol or higher alcohols → Concentration of extract → Removal of sugars, fats and colours → Isolation of crude aloin → Purification of aloin.

Crystallization/drying: To carry out thin layer chromatography pre coated silica gel plates were used (Merck-Ag). Genesys 10 UV scanning spectrophotometer is used for spectrophotometric assay.

Nitrogen is estimated by Kjeldahl method. The amount of sample taken is 0.1944 g, 0.3 g of $CuSO_4$, 10 g of NaOH (to prepare 0.1 N solution) and 25 mL of H_2SO_4 were used. Sodium hydroxide solution is standardized by titrating against potassium hydrogen phthalate. Sulphuric acid of 0.1 N concentration is prepared and standardized by titration with standard sodium carbonate.

Ash content: 1 g of the ground drug is incinerated in a tarred platinum or silica dish at a temperature not exceeding 450 °C until free from carbon. Then it is cooled and weighed. The percentage of ash is calculated with reference to the dried drug. The procedure carried out protected from light.

RESULTS AND DISCUSSION

Aloin is a crystalline substance with yellow colour extracted from Barbados aloes or cape aloes or *Aloe barbadensis* Miller. It is mainly obtained in the resin column during the powder preparation and processed. It contains not less than 70 % anhydrous barbaloin (10-β-glucopyranosyl-1-1,8-dihydroxy-3-hydroxymethyl anthracen-9-one), a glucopyranosyl derivative of aloe-emodin anthrone, calculated with reference to the dried substance. It is an yellow crystalline powder having slight odour of aloe to odourless. It is almost completely soluble in 130 parts water, in ethanol 96 % and very slightly soluble in chloroform and ether. Some other properties of aloin is given in Table-1.

The mixture is subjected for thin layer chromatography (TLC). The mobile phase includes 100 volumes of ethyl acetate, 17 volumes of methanol and 13 volumes of water.

Solution (1): In 20 mL methanol, 0.5 g of test substance is heated. Then the solution is shaken for few minutes and then decanted. The supernatant liquid is maintained at 4 °C and used within 24 h.

Solution (2): 50 mg of barbaloin (10-β-glucopyranosyl-1-1,8-dihydroxy-3-hydroxymethyl anthracen-9-one). It's

TABLE-1
PROPERTIES OF ALOIN

Test	Procedure	Observations
Characteristics	Visual Inspection	➢ A yellow to greenish yellow coloured crystalline/free flowing powder. ➢ Slight odour characteristic of Aloe to odourless.
Solubility	Quantitative test	➢ Almost completely soluble in 130parts water. ➢ Soluble in ethanol 96 %. ➢ Very slightly soluble in chloroform and Ether.
Identification	1. Thin Layer Chromatography (TLC) 2. Chemical identification a. Reaction with Sodium Tetra-borate solution b. Reaction with Bromine water	1. R_f value 0.4-0.5. Compared with the B.P. standard. 2. Fluorescence in UV light (365nm) Copious yellow precipitate. (IR spectrum in nujol corresponds to that of reference standard)
Acidity	pH of 1% w/v suspension	pH is 4 to 6.5
Light absorption	By UV spectrophotometry	In the range of 250 to 370 nm of a freshly prepared solution exhibits maximum at 266, 298 and 354nm
Water insoluble matter		Not more than 1.5 %
Loss on drying		Not more than 5 % (60 ºC 3 h)
Assay	By UV/VS spectrophotometry	Not less than 70 % of anhydrous aloin

molecular formula is $C_{21}H_{22}O_9$ and molecular weight is 418.4, it is an lemon yellow to dark yellow needles or crystalline powder. It darkens on exposure to Sunlight, is dissolved in 10 mL methanol. After removal of plate it is allowed to dry in air, then the plate is sprayed with 10 % w/v solution of potassium hydroxide in methanol and examined ultraviolet light at 365 nm. Chromatograms obtained with solution (2) showed a yellow band with R_f value of 0.4 to 0.5. The chromatogram obtained with solution (1) showed yellow band corresponding to that obtained with solution (2).

Identification of B: 10 mL of 0.2 % w/v solution is heated with 0.1 g of sodium tetraborate in water bath for 5 min. 2 mL of the resulting solution is poured into 20 mL water. A yellowish green fluorescence is produced which is particularly marked by ultraviolet light.

Identification of C: 1 mL bromine water is added to 5 mL of 2 % w/v solution. A copious yellow precipitate is formed.

Acidity: pH of 1 % w/v suspension varied between 4 to 6.5 %.

Light absorption: In a range of 250 to 370 nm, a freshly prepared 0.0025 % w/v solution exhibited maximum at 266, 298 and 354 nm.

Water insoluble matter: Not more than 2 % when determined by the following method: To 1 g of the compound, 120 mL of water is added. The solution is shaken frequently for 2 h, maintaining the temperature at 25 °C. Then it is filtered through a sintered glass filter (used B.S. porosity No. 2). The residue is washed with 25 mL of water and dried to a constant weight at 70 °C and a pressure of 2 kPa for 3 h.

Loss on drying: When dried at 70°C at a pressure of 2kPa for 3 h, loses not more than 5 % of its weight. The ash not more than 0.5 %.

Assay by UV spectrophotometry: 2 mL of NaOH, 0.2 g of aloin, 100 mL of boiled and cooled water, 10 mL CCl_4, 0.6 g of $FeCl_3$, 6 mL HCl are intimately mixed and cooled. Blank calibration was performed with 0.1 N NaOH. Wavelength ranges chosen were 195 to 600 nm.

Concentration of aloin: (devised formula) = (Sample absorbance/standard absorbance × standard weight/sample weight) × 88 {88 is the standard aloin concentration}

Aloin used for this test has been acquired in the resin column (filtered, isolated and purified from crude form). Varying levels of concentrations were seen in the aloin content of soil grown *Aloe barbadensis* Miller plant and the *in vitro* cultured plants that were grown on chemically altered MS callus induction medium.

Graphical reports for both these samples BLE-I and BLE-II exhibit the following results.

BLE-I (*in vitro* cultured *Aloe barbadensis* Miller plant) → 96.19 %.

BLE-II (field grown *Aloe barbadensis* Miller plant) → 77.23 %.

After the process of Kjeldahl digestion and extraction, the resultant fraction of liquid is collected (tapping acid along with ammonia) and subjected to titration.

Formula for calculating the protein content present in the sample:

$$\frac{\text{Protein factor}}{\text{Sample weight}} \times \frac{\text{Titer value} \times \text{Normality}}{100} = \text{Total protein content (\%)}$$

Protein factor: 875 (constant)

Calculation for field grown *Aloe barbadensis* Miller plant: Weight of sample taken was 0.1944 g, T1 (initial burette reading) = 48.9; T2 (final burette reading) = 43.8, Titer value (T1 - T2) = 5.1, Normality = 0.1018 N

$$\frac{875}{0.1944} \times \frac{(48.9 - 43.8) \times 0.1018}{100} = 23.3\%$$

Nitrogen factor = 6.25

To calculate the amount of nitrogen present in the given sample, the percentage of protein should be divided by the nitrogen factor which is a constant. *i.e.*,

$$\frac{\text{Protein (\%)}}{\text{Nitrogen factor}} = \text{Nitrogen (\%)}$$

$$\frac{23.3}{6.25} = 3.728\ \%$$

Permissible amounts of nitrogen in the aloe vera plant is 3 to 4%.

Calculation for *in vitro* cultured *Aloe barbadensis* Miller plant: Accurate weight of sample: 0.2078 g, T1 (initial

burette reading) = 50, T2 (final burette reading) = 45.8, Titer value (T1 - T2) = 3.8, Normality = 0.1018 N

$$\frac{875}{0.2078}\times\frac{(50-45.8)\times 0.1018}{100}=18.01\%$$

Nitrogen factor = 6.25

To calculate the amount of nitrogen present in the given sample, the percentage of protein should be divided by the nitrogen factor which is a constant.

$$\frac{\text{Percentage of protein}}{\text{Ntrogen factor}}=\text{Per cent of nitrogen}$$

$$\textit{i.e.},\quad \frac{18.01}{6.25}=2.081\ \%$$

The results indicate that the aloin is a polar compound and mild acid. It has aromatic nucleus with unsaturation in the molecule. It has very less quantity of water insoluble matter. Successfully *in vitro* cultured *Aloe barbadensis* Miller has shown considerable high yield of aloin concentration when analyzed after a growth period of 65 days approximately. It has been inferred that *in vitro* cultured aloe plants have shown much lesser quantity of nitrogen and is a good sign of improved quality of the species when compared to the mother plantation grown in the field (soil rich in minerals and the sample has been taken from all the plants grown in the same area).

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REFERENCES

1. B.K. Vogler and E. Ernst, *Br. J. Gen. Pract.*, **49**, 823 (1999).
2 K. Eshun and Q. He, *Crit. Rev. Food Sci. Nutr.*, **44**, 91 (2004).
3 F. Rodriguez, H. Baldassarre, J. Simonetti, F. Aste and J.L. Ruttle, *Theriogenology*, **30**, 843 (1988).
4 W.G. Wamer, P. Vath and D.E. Falvey, *Free Radic. Biol. Med.*, **34**, 233.
5 M.D. Boudreau and F.A. Beland, *J. Environ. Sci. Health, Part C*, **24**, 103 (2006).