

EXPERIMENT NO.11

Aim: To extract total Sennosides from senna leaves and identify by Thin Layer Chromatography Method (TLC).

Requirements

1) Apparatus: Beaker, Stirrer, Test tubes, Measuring Cylinder.

2) Chemicals: Senna leaves, benzene, methanol,

HNO_3 calcium chloride, potassium hydroxide.

THEORY-

It is obtained from the dried leaflets of *Cassia angustifolia*, *Cassia auriculata* belonging to

family Leguminaceae (Casalphenaceae). Sennosides are the anthraquinone glycosides. Senna glycoside, also known as sennoside, is a medication used to treat constipation and empty the large intestine before surgery. The medication is taken by mouth or via the rectum. It typically begins working in minutes when given by rectum and within twelve hours when given by mouth. It is a weaker laxative than castor oil.

EXTRACTION PROCEDURE:

The weighted quantity of dried powdered senna leaves was extracted with benzene for 30 min on electric shaker, filtered in vacuume and benzene gets distilled off. The left over marc was dried at room temperature and extracted with 70% methanol for three hrs. and filtered under vacuum. The marc was re-extracted with 50 ml of 70 % methanol for 15min, Filtered and the methanolic extracts get combined. The methanolic extract was concentrated to 1/4th volume, acidified to pH 3.2 by adding HNO_3 with constant stirring. It was set aside for 15 min. at 5°C , filtered and 1g of anhydrous calcium chloride in 10 ml of denatured spirit was added with constant stirring. The pH of the solution was adjusted to 8 by addition of potassium obtained in as calcium sennoside dried and weighed. The percentage yield was also calculated.

General conformation tests for sennosides:

Bornstager's Test

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Calculation

$$\begin{aligned} V. \text{ Yield} &= \frac{2.60}{50} \times 100 \\ &= 5.2\% \end{aligned}$$

$$\begin{aligned} R_f \text{ value} &= \frac{4.6}{53} \\ &= 0.66 \end{aligned}$$

(50)

To 3 ml sample, dilute sulphuric acid (H₂SO₄) was added, boiled and filtered. To the cold filtrate, equal volume of benzene was added and shaken. The organic solvent was separated and ammonia was added. It was observed that the ammoniacal layer turned pink confirming the presence of anthraquinone glycoside presence.

Modified Bomtager's Test

To 5 ml sample, 5 ml Ferric chloride and 5ml dilute hydrochloric acid was added. It was then

heated for 5min in boiling water bath. Cooled and benzene was added. It was shaken well,

separated and equal volume of dilute ammonia was added. It was observed that the

ammoniacal layer turned pink confirming the presence of anthraquinone glycoside.

IDENTIFICATION BY TLC

Sennosides is identified by thin layer chromatography by using silica gel GF as stationary phase

and n-propanol: ethyl acetate: water: glacial acetic acid (40:40:29:1) as mobile phase

After drying the TLC plate, Sennosides is detected at a particular R_f value after spraying

reagent using HNO₃-KOH reagent.

RESULT-

The percentage yield of sennoside from senna leaves was found to be 5.24.

The R_f value of extract of sennoside from senna leaves was found to be 0.86