

Quantitative Analysis of Secondary Metabolites of Withania Somnifera and Datura Stramonium

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Abstract: Medicinal plants are importance resource of drugs of traditional system of medicine. Secondary metabolites are the chemical constituents present in the plants and are important in determining medicinal properties of the plant. The present paper deals with the methanol extract, n - Hexane extract of Withania somnifera, Datura stramonium were screened for the quantitative analysis by standard procedure and subjected to analysis by UV Spectrophotometer. Results shows that Steroids and Alkaloids are in higher amount than the phenols & flavonoids. This indicate that seasonal variation played a key role in increasing secondary metabolites rather than the phenological stage of the plant.

Keyword: Withania somnifera, Datura stramonium Secondary metabolites qualitative analysis

1. Introduction

The medicinal values of the plant lies in chemical substances that produces a definite physiological action on human body most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Medicinal plants shows a marked variation in active ingredients during different season these have been widely attributed to variation in environmental variables such as temperature and rainfall (Reddy & Reddy, 1997 Ghimire et.al. 2008:2009) In addition there is no knowledge in local harvester for choosing an appropriate time for harvesting to maximize the medicinal potency of the plants such as withania somnifera, Datura stramonium. Production of secondary metabolites is organ/stage specific and depend largely on the physiological stage of the plants. (Acamovic & Brooker 2005) Therefoialre efforts have always been made to increase the metabolic contents of secondary metabolites in Withania somnifera & Datura stramonium.

2. Materials

Fresh plant part in this investigation were collected from experimental plots (4*2 m each) of the JSM college Alibag. Fresh plant material were washed under running tap water then air dried under shade after complete shade drying the plant material was grinded. The powder was kept in small plastic bags with paper labelling.

Methanol extraction: The dried plant parts of Withania somnifera and Datura stramonium were grinded. 5 gm of both the crude herb were weighed and extracted with 50 ml methanol at 70⁰ C for 1 hour. Yield of extract obtained from crude herb powder of Withania somnifera is 30 % and Datura stramonium is 35 %.

n Hexane extraction: The dried plant parts of Withania somnifera & Datura stramonium were grinded. 5 gm of both the crude herb were weighed and extracted with 50 ml methanol at 70⁰ C for 1 hr. Yield of extract obtained from crude herb powder of Withania somnifera is 10 % while Datura stramonium is 15 %. Methanol extract was used for Total flavonoids, Phenols and Total alkaloid estimation

where as n Hexane extract was used for total steroids estimation.

3. Methods

The plant samples of Withania somnifera, Datura stramonium were analysed for flavonoids, phenolic contents, Total alkaloids, steroids by using UV Spectrophotometer(V 360). Following methods were adopted for analysis.

Total Flavonoids Content

Preparation of Standard:

- Weigh 10 mg of Quercetin standard in 50 ml of volumetric flask and dilute up to the mark with methanol (200µg/ml) (stock solution).
- From the stock solution, prepare solutions of 4, 8, 12, 16, 20 µg/ml with methanol.
- 1 ml standard was taken and transferred in a test tube.
- Add 0.1 ml of 1M potassium acetate solution in each flask. After 6 minutes add 0.1 ml of 10% AlCl₃ solution. Mix well.
- 2.8 ml of Distilled water was added Mix well.
- Incubate the solution for 30 mins. Determine the absorbance at 415 nm.

Preparation of Sample:

- 2.5 ml sample extract in methanol (liquid sample- 1mg/ml) was taken and transferred in 10ml volumetric flask dilute it up to the mark with methanol.
- 1 ml of this solution was taken and transferred in a test tube.
- Add 0.1 ml of 1M potassium acetate solution in each flask. After 6 minutes add 0.1 ml of 10% AlCl₃ solution. Mix well.
- 2.8 ml of Distilled water was added Mix well.
- Incubate the solution for 30 mins. Determine the absorbance at 415 nm.

Observations

Conc. µg/ml	Absorbance
4	0.1101
8	0.1669
12	0.2348
16	0.3201
20	0.3865
<i>Datura Metel</i>	0.2205
<i>Withania Somnifera</i>	0.1263

Sample	Absorbance	Content (mcg/ml)	Content*DF (mcg/ml)	Content (mg/ml)	% content Flavonoid in extract	% content Flavonoid in crude herb
<i>Datura Metel</i>	0.2205	11.147	44.588	0.0446	4.458%	1.56%
<i>Withania Somnifera</i>	0.1263	5.606	22.424	0.0224	2.242%	0.673%

4. Total Phenolic Content

Preparation of Standard:

- Weigh 25 mg of Gallic acid in 25ml volumetric flask and dilute upto the mark with Methanol. (Stock solution)
- From the stock solution, prepare 5,10,15,20 and 25 µg/ml with methanol.

Preparation of Sample:

- Take 1 ml of each standard prepared (5, 10, 15, 20 and 25 µg/ml) and 1 ml sample (liquid sample 1mg/ml) in each 10 ml volumetric flask separately and dilute it with methanol.
- 1 ml sample was taken and transferred in a test tube.
- Add 1 ml of Folin-Ciocalteu reagent in each volumetric flask.

- Allow to stand for 5 mins.
- Add 1 ml of (10% Na₂CO₃) in each 10 ml volumetric flask.
- Make up the volume to 10 ml with distilled water.
- Allow to stand for 30 mins.
- Take reading at 730 nm.

Observations

Sr. no.	Conc (mcg/ml)	Absorbance
1	0.5	0.1137
2	1.0	0.3332
3	1.5	0.5435
4	2.0	0.7689
5	2.5	0.9684
6	<i>Datura Metel</i>	0.1775
7	<i>Withania Somnifera</i>	0.2010

Sample	Absorbance	Content (mcg/ml)	Content*DF (mcg/ml)	Content (mg/ml)	% content Phenol in extract	% content Phenol in crude herb
<i>Datura Metel</i>	0.1775	0.642	6.42	0.00642	0.642%	0.224%
<i>Withania Somnifera</i>	0.2010	0.697	6.97	0.00697	0.697%	0.209%

5. Estimation of Total Alkaloid Content

Preparation of reagents:

- Bromocresol green solution was prepared by heating 69.8 mg Bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water.
- Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2M sodium phosphate (71.6 gm Na₂HPO₄ in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 gm citric acid in 1 L distilled water).
- Atropine standard solution was made by dissolving 1 mg of pure Atropine in 10 ml distilled water.

Preparation of Standard Curve

- Accurately measured aliquots (0.2, 0.4, 0.6, 0.8, 1 and 1.2 ml) of Atropine standard solution was transferred to different separating funnels.
- Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform.
- The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform.
- The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer against the blank prepared as above but without Atropine.

Separation of Alkaloid

- 50mg of extract was dissolved in 100 ml of 2N HCL and then filtered. 50ml of this solution was transferred to separating funnel.
- Then add 5 ml of BCG solution and 5 ml of phosphate buffer (pH=4.7) were added to this solution.
- The mixture was shaken and complex extracted with 2ml-2ml batch extraction chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform.

Observation Table

Concentration (mcg/ml)	Absorbance (at 470 nm)
2	0.0659
4	0.0789
6	0.1062
8	0.1278
10	0.1441
12	0.1662
<i>Datura Metel</i>	0.0785
<i>Withania Somnifera</i>	0.0713

Sample	Abs	Content (mcg/ml)	Content*DF (mcg/ml)	Content (mg/ml)	% content Alkaloid in extract	% content Alkaloid in crude herb
<i>Datura Metel</i>	0.0785	3.55	71	0.071	7.1%	2.485%
<i>Withania Somnifera</i>	0.0713	2.83	56.6	0.0566	5.66%	1.715%

6. Total Steroids Content

Standard Preparation:

- Take 50 mg of Lupeol in chloroform and make up the volume up to 50 ml with chloroform. (1000 µg/ml).
- From the above solution take 20 ml and make up volume up to 50 ml with chloroform. (400 µg/ml)
- From the above solution take 6, 7, 8, 9 and 10ml evaporate to dryness.
- In that each add 0.5 ml Glacial acetic acid, 5 ml Liebermann Burchard's reagent make up the volume up to 10 ml with chloroform, keep it for 20min.
- See the absorbance at 618nm.

Preparation of Sample (Non Hydrolysed):

- 25 mg of n-hexane extract was diluted to 25ml in volumetric flask using chloroform.
- Filter the solution (**Stock solution**)
- 1ml chloroform extract (Stock solution) evaporate to dryness then add 0.5 ml Glacial acetic acid and 5 ml Liebermann Burchard's reagent, make up the volume up to 10 ml with chloroform.
- Keep it for 20 min and see the absorbance at 618 nm.

Blank preparation:

Add 0.5 ml Glacial acetic acid and 5 ml Liebermann Burchard's reagent; and make up the volume up to 10 ml with chloroform.

Observations

Sr. no.	Conc. mg/ml	Absorbance
1	2.4	0.1545
2	2.8	0.2005
3	3.2	0.2328
4	3.6	0.2563
5	4.0	0.2775
6	<i>Datura Metel</i>	0.1699
7	<i>Withania Somnifera</i>	0.1829

Sample	Absorbance	Content	Content*DF(mg/ml)	%	%
<i>Datura</i>	0.1699	2.492	0.249	24.92%	2.492%
<i>Withania</i>	0.1829	2.665	0.266	26.65%	2.665%

7. Results

	<i>Datura Metel</i>	<i>Withania Somnifera</i>
Total Flavonoid	1.56%	0.673%
Total Phenol	0.224%	0.209%
Total Alkaloid	2.485%	1.715%
Total Steroid	2.492%	2.665%

8. Discussion

Presence of phytochemicals contribute medicinal as well as physiological properties to the plant which used in treatment of different ailments. Phytochemicals analysis is very

important in the evaluation of active biological components of medicinal plants. The quantitative estimation was carried out by using UV spectrophotometer. one can easily know about the medicinal potency of plants by using this technique. The estimation were carried out with the help of methanolic extract & n-Hexane extract of plant material. In the leaves extract of *Withania somnifera* the amount of total steroids (2.665 %) and amount of total alkaloids (1.715 %) are higher than the other chemicals. In *Datura stramonium* leaves extract the amount of steroids (2.492 %) are higher than the alkaloids (2.485 %). The leaves extract shows abundant alkaloids and steroids while phenolic compounds & flavonoids are in trace amount.

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