

Quantitative Analysis of Secondary Metabolites of Withania Somnifera and Datura Stramonium

Pralhad D Naik¹, Mishra R L²

Department of Botany JSM College Alibag, District - Raigad Maharashtra (India)

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.

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.

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.

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.

References

- [1] African journal of biotechnology 2008, 7(12),1797-1806.
- [2] Rastogi R P & Mehrotra B N compendium of indian medicinal plants (CDRI, Lucknow & publ & information Directorate New Delhi) 1991.
- [3] Jain J-P, A clinical trial of kantakari (*Solanum xanthocarpum*) in case of casa raga, J Res Ayur siddha, 1 (1980), 35-51.
- [4] Dubey p & Gupta p c, A new flavonal glycoside from the flowers of *S. xanthocarpum*, phytochemistry, 17 (1978) 2138.
- [5] Gupta m p & Datta s. the chemical examination of *Solanum xanthocarpum* scharf & wendel. part I.
- [6] Khandelwal k.r. practical pharmacognosy techniques & Experiments, Edn 8, Nirali prakashan publication 2001.
- [7] Harborne J B phytochemical methods - A guide to modern techniques of plant anlysis Edn 3, Chapman & Hall publications 1998.
- [8] Kokate c.k. practical pharmacognosy, vallabh prakashan publications, New Delhi 2002, 103-107.
- [9] Indian pharmacopoeia (2007) Indian pharmacopoeia commission Ghaziabad, pp2037-2038.
- [10] The ayurvedic pharmacopoeia of india (2001) 1st edn, pp 41-42.
- [11] Patwardhan B Gautam M (2005) Drug Discov. Today 10; 495-502.
- [12] Rout k. k. pradhan s, mishra s k (2008) J AOAC 91:1149.
- [13] Kirtikar K R Basu B D. 1993 Indian medicinal plants Vol. I Allahabad: lalit mohan Basu; 1993. p.195.
- [14] Trivedi pc. 2002 Ethnobotany. Jaipur. Aavinashkar publisher and distributors, p. 222.
- [15] Chatterjee A, pakrashi sc. The treatise on indian medicinal plant 1995; 4: 208-212.
- [16] Singh N, nath R, Lata A et.al. *Withania somnifera* (ashwagandha) rejuvenating herbal drug which enhances survival during stress (an adaptogen) Int J crude Drug Res 1982 ; 20 :29 - 35.
- [17] Rastogi R P Mehrotra B N. compendium of indian medicinal plants, vol. 6. Central Drug research institute. New Delhi. 1998.

- [18] Bhattacharya s k satyan k s chakraborti A. Effect of trasina an ayurvedic herbal formulation.
- [19] Malhotra CL, Mehta VL, Das PK Dhalla NS. studies on Withania ashwagandha, kaul.v. The effect of total alkaloids on central nervous system. Indian J of physiol pharmacol 1965 ; 9:127-136.
- [20] Bennet, P. N. & M. J. Brown. 2003, Clinical Pharmacognosy; Ninth Edition, 264-267.
- [21] Khan. T. Zahid M. Asim, S H.Z; Iqbal, M. Iqbal, C & V.A. Udin.2002; Pharmacological activities of crude acetone extract wall. Phytomedicine, 9, 749-752.
- [22] Rajesh D H., Singh, A. H., Chillar, M. and G L Sharma 2004.
- [23] Chopra R. N; Nayar. S. L. and I.C. Chopra 1956.Glossary of Indian Medicinal plant Council of scientific and Industrial Research (CSIR), Publication and Information Directorate, New Delhi, 57, 102, 139, 198 & 256.
- [24] Tiwari, R and B K Singh. 1943 “Chemical examination of *Cassia alata* L. Part – I
- [25] Zargari, A, 1990; Medicinal Plant; Fourth Edition. Tehran University Publication; 42 - 45.
- [26] Mehra 1986, Sharma 1998, Chauvan 1999 - Plant drugs.
- [27] Yaniv& Palvitch 1982, Serine et.al. 1982. A report on variation in quinine.
- [28] Surenge et al., 1973; Chandramouli, 1976; Variation in diuretic activity & alkaloidal content of *Boerhavia diffusa* root.
- [29] Bose P.K., Banerje, A.K. Ghosh, C. (1953-1955). Chemical investigation of *Paederia foetida*. Transaction of Bose Research Institute, Calcutta, 19:77.
- [30] Tandon, M.; Shukla, Y.N.; Thakur, R. S. (1990). Steroids, glycosides from *Asparagus scenedesmus*, Phytochemistry, 29(9): 2957- 295.
- [31] Kokate C.K, Purohit A.P and Gokhale S.B., Practical Pharmacognosy; 2nd edition. Vallabh Prakashan, New Delhi, 2004, 466-470.
- [32] Horbone, J.B., In: Phytochemical methods, 2nd edition. Chapman and Hall, New York,1984. Kokate C.K, Purohit A.P and Gokhale S.B., In: Pharmacognosy, 3rd edition. Niralin Prakashan, Pune. 1995.
- [33] Chung K.T, Wong T.Y, Wei C.L, Huang Y.W, Lin Y., Tannins and human health: a review. Criti. Rev. Food. Sci. Nutri., 1998, 6,421-64.
- [34] Asthana, R. and Raina, M. K. (1989): Pharmacology of *Withania somnifera* (L.) Dunal - a review, Indian Drugs, 26(5): 199-205.
- [35] Nadkarni, A. K. (1954): Dr. K. M. Nadkarni’s Indian Materia Medica, Third edition, Two volumes, Popular Book Depot, Bombay, Dhootapapesheshwar Prakashan Ltd., Panvel.
- [36] Sharma K and Dandiya PC, *Withania somnifera* Dunal - present status. Indian
- [37] Tripathi AK, Shukla YN and Kumar S, Ashwagandha (*Withania somnifera*-Dunal (Solanaceae): A status report. J Med Arom Plant Sci, 18; 46-62 (1996). drugs, 29: 247- 253 (1992)