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Comparative UV-Spectroscopic and Chemical estimations of Atropine Alkaloid present in Datura stramonium found in Saudi Arabia

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Abstract

Aim: *To find out the concentration of Atropine alkaloid in Datura stramonium of Saudi Arabian Region and to develop a new method of the Atropine estimation.*

Methods: *The presence of Atropine in the alcoholic extract of leaves of Datura stramonium was known by Qualitative TLC profile. Quantitative estimation of Atropine in the extract was done with UV Spectrophotometric Method (new proposed method for determination of Atropine in plant extract) and Titrimetric Method (Pharmacopoeial method) and results were compared.*

Results: *Qualitative (TLC) Result showing the presence of Atropine in the Datura stramonium extract. After applying the calculations the concentration of Atropine was found to be 0.3% (w/w). Since the both quantitative results were comparable and the values of the results were approximately equal supporting the validation of New Method.*

Conclusion: *The Saudi Arabian geographical variety of Datura stramonium contains the Atropine Alkaloid as 0.3% (w/w) which is close to the reported value. The new method for the quantitative estimation of Atropine i.e. UV spectroscopic method was developed successfully.*

Keywords: Datura stramonium, Saudi Arabian Region, Atropine, Quantitative estimation, UV Spectrophotometric Method

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1. INTRODUCTION

General Introduction:

Medicinal Plants are of very much importance to human health. They provide the crude drugs which are useful in various diseases. The crude drugs are the sources of various phytoconstituents which are reported to be active for various pathological conditions. These conditions are diabetes, hypertension, hepatotoxicity, cancer, arthiritis, oxidative stress etc. The concentration and composition of phytoconstituents varies according to geographical location [(1)]and the medicinal Value depends on the concentration and composition of the active ingredients [(2)]

Many Phytochemists who are working on phyotoconstituentsof medicinal plants are also trying to do comparative studies of a particular active constituent [(1)] in various geographical varieties of Medicinal Plants.

PURPOSE OF THE PRESENT STUDY

Atropine is the alkaloid (phytoconstituent) which is obtained from *Datura stramonium* (Solanaceae) which is of wide universal occurrence [(3)] It may differ in the Atropine quantity based on its geographical location [(1,3)] The purpose of the study was to estimate the quantity of Atropine in *Daturastramonium* of Saudi Arabian location. The two methods, UV spectroscopic Method and chemical method were employed to see the accuracy in the result as well as to establish UV spectroscopic Method as the new method for quantitative estimation of Alkaloid.

Phytoconstituents:

The phytoconstituents obtained from the plants include primary metabolites as well as secondary metabolites. The primary metabolites are Carbohydrates, Lipids, Proteins and Nucleic Acids etc. while the secondary metabolites are Alkaloids, Glycosides, Tannins, Resins, Terpenoids and

Flavanoids etc. Secondary metabolites can be observed through physical as well as chemical analysis. Growth and development Medicinal plant and their metabolites are affected by physical environment, including light, temperature, rainfall and soil properties. (3)

Analysis of Phytoconstituents:

Now a day a lot of techniques are available for the qualitative as well as quantitative analysis of Phytoconstituent. The chromatographic techniques include HPLC, HPTLC, TLC, Column Chromatography etc. while the spectroscopic techniques include UV, IR NMR and Mass Spectroscopies etcThe qualitative chemical tests also done with the help of various chemical reagents. These tests include various color reactions as well as precipitate reactions. (3)

About the Alkaloids (3)

i) Definition

Alkaloids, which mean alkali-like, are basic nitrogenous compounds of plant or animal origin. They are physiologically active.

ii) Occurrence

The major source of alkaloids is the flowering plants (Angiosperms). Recently, alkaloids are found in animals, insects, fungi and marine organisms also.

iii) Availability:

They occur in the form of salts with organic or inorganic acids, or in combination with specific acids. e. g. opium alkaloids occur with meconic acid

iv) Function of alkaloids in plants:

1. Protective.
2. As end products.
3. Waste products.
4. Source for energy and reserve of nitrogen

v) Physical characters

Most alkaloids are crystalline solids, some are liquids which are volatile e.g. nicotine and anabasine. Others are non-volatile liquids e.g. hyoscine. They are mostly colorless, some are colored. e. g. canadine is orange.

vi) Solubility

A- Alkaloidal Bases

Generally, alkaloidal bases are insoluble in water but soluble in organic solvents. Some are soluble in H2O e.g., Ephedrine, Caffeine, Colchicine, Codeine, Pilocarpine, and quaternary bases e.g. d-Tubocurarine. Alkaloidal bases are sparingly soluble in organic solvents e.g. morphine is sparingly soluble in ether. Theobromine and Theophylline are insoluble in benzene.

B- Alkaloidal salts

These are soluble in H_2O, sparingly or insoluble in organic solvent. Some are insoluble in H_2O e.g. quinine sulfate.Some are soluble in certain organic solvents e.g. lobeline HCl and apoatropineHCl are soluble in $CHCl_3$. Generally, salts of weak bases are easily hydrolyzed in solution and can be extracted with organic solvents e.g. colchicine is soluble as its base or HCl in H_2O and chloroform.

vii) Nitrogen in alkaloids

Alkaloids usually contain one nitrogen atom either in a heterocyclic ring or in the side chain.- Some alkaloids occur as primary amines e.g. (+) - nor pseudoephedrine Secondary amines e.g. (-) - ephedrine. - Tertiary amines e.g. nicotine, atropine, and most alkaloids -Quaternary bases e.g. (+) - tubocurarine.

viii) Qualitative Analysis of alkaloids

Generally, alkaloids can be detected by two groups of reagents.

1. The group give amorphous or crystalline precipitates.
2. While those give characteristic colors with alkaloids.

Alkaloidal precipitants:

Most alkaloids are precipitated from neutral or acidic solutions by a number of reagents which contain certain heavy metals such as mercury (Hg), platinum (Pt), bismuth (Bi) and gold (Au), by forming double salts with them. The common alkaloidal precipitants are:

1. Mayers Reagent
2. Wagners Reagent
3. Hagers Reagent
4. Dragendorffs Reagent

Alkaloidal colour reagents:

They give characteristic colors with most of the alkaloids. Most of these reagents contain H_2SO_4. This group of reagents is usually applied to the alkaloids themselves and not to their solutions. The most common alkaloidal color reagents are:

1-Froehd's reagent

(H2SO4 + ammonium molybdate).

2. Mandalin's reagent

(H2SO4 + ammonium vanadate).

3. Marquis reagent

(H2SO4 + few drops of formaldehyde).

4. Erdman's reagent

(H2SO4 + HNO3)

ix) Quantitative Analysis of Alkaloid: (3)

For quantitative estimation of alkaloids, they must be first extracted. Generally Chloroform is used as a solvent for extraction. It should not be evaporated to dryness because some $CHCl_3$ may be decomposed and form the hydrochloride of the alkaloids. Therefore, $CHCl_3$ should be evaporated to a small volume and then neutral ethanol is added and evaporated to dryness. The purified residue, which remains after evaporation of the solvent, consists usually of a mixture of alkaloids.

For determination of each of the individual alkaloids, they must be first separated. The assay aimed to determine the individual alkaloid is called ultimate assay. Assays for determining total alkaloids are called proximate assays.

2. REVIEW OF LITERATURE

Introduction:

Datura stramonium is a widespread annual plant from the Solanaceae family. It is one of the widely well knownfolklore medicinal herb. It is a wild growing flowering plant and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom (N-CH3) and include the anti-cholinergic drugs atropine and scopolamine. An extract made from the leaves is taken orally for the treatment of asthma and sinus infections and stripped bark are applied externally to treat swellings, burns and ulcers. (5)

Figure 1: Datura stramonium Plant

Biological Source:

Datura consists of dried leaves and flowering tops of Daturastramonium Linn belonging to family solanaceae . The drug is required to contain not less than 0.25% of alkaloid calculated as Hyoscyamine. Prepared Stramonium is the finely powdered drug adjusted to an alkaloid content of 0.23-0.27%. (3)

Geographical Source:

Datura stramonium is found widely in European, Asian, and other countries and in South Africa. The plant grows commonly in waste places throughout India from Kashmir,Malabar. It is cultivated in Germany, France, Hungary and South America (3), The species of Datura can be found throughout the world. The plant grows in sandy flats, plains, areas up to 2,500 feet above sea level. The origin of Datura stramonium is disputed. The Sanskrit dhattura and the Hindustani dhatur formed the basis of the general name. The origin of Jimson weed could be Asiatic. Some sources report a probable Central American origin, due to Datura's habitation of most temperate and subtropical parts of the world. (6)

Chemical constituents:

Plant contains 0.2- 0.6% alkaloids. The main alkaloids are Hyoscyamine and Hyoscine)Scopolamine(as discussed above. It also contains protein albumin and Atropine. Atropine is formed from hyoscyamine by racemisation. These alkaloids are usually present in the proportion of about two parts of Hyoscyamine to one part of Hyoscine, but in young plants Hyoscine is predominant alkaloid(7) .

Phytochemical Investigations:

Beside major tropane alkaloids like Hyoscyamine and Scopolamine, several minor tropane alkaloids have been identified in Datura species. Typical examples of minor alkaloids in D. stramonium are Tigloidin, Aposcopolamine, Apoatropin, Hyoscyamine N-oxide and scopolamine N-oxide ditigloyloxytropane and 7-hydroxyhyoscyamine are reported for the first time in this species. (8) Distribution of Hyoscyamine and Scopolamine in D. stromonium was studied. The production of Hyoscyamine and Scopolamine in D. stromonium has been investigated in the different plant parts, at

different stages of their life cycle. The maximum contents were found in the stems and leaves of young plants, Hyocyamine being always the predominate component. These compounds were included in many pharmacopieasbecause of their anticholinergic activities. (8)

Pharmacological Investigations:

The Datura has many of pharmacological activities such as :

Antiasthmatic activity: *Datura* in asthma treatment and possible effects on prenatal development was reported by Pretorius and Marx in 2006 (9)

Anticholinergic activity: The alkaloids found in *Datura* , are organic esters used clinically as anticholinergic agents. Symptoms of acute weed poisoning included dryness of the mouth and extreme thirst, dryness of the skin, pupil dilation and impaired vision, urinary retention, rapid heartbeat, confusion, restlessness, hallucinations, and loss of consciousness .(10)

Antimicrobial Activity: The methanol extracts of *D. stromonium* and *Datura inoxia* showed activity against Gram positive bacteria in a dose dependent manner.(11)

Anticancer activity: Thousands of herbal and traditional compounds including *Datura stramonium* screened worldwide to validate their use as anti-cancerous drugs, was used to cure cancer. (12)

3. AIMS AND OBJECTIVES

- Atropine is the alkaloid (phytoconstituent) which is obtained from Datura stramonium (Solanaceae) which is of wide universal occurrence (3)
- It may differ in the Atropine quantity based on its geographical location (1,3)
- The purpose of the study was to estimate the quantity of Atropine in Datura stramonium of Saudi Arabian location.
- The two methods, UV spectroscopic Method and chemical method were employed to see the accuracy in the result as well as to establish UV spectroscopic Method as the new method for quantitative estimation of Alkaloid.

4. MATERIALS AND METHODS

Instrumentation

UV-Visible Spectrophotometer Double beam (model-UV-1650PC Shimadzu, Japan) with matching quartz covet was used to measure absorbance.

Chemicals

Authenticated marker (Standard Atropine alkaloid) compound was used for the method development. All chemicals used were of analytical grade.

These chemicals include acetic anhydride, Per chloric acid, Glacial acetic, Crystal violet indictor, Dragendorrf Reagent etc.

Apparatus Used:

Soxhlet apparatus

Glassware:

Measuring cylinder, pipette, funnel, test tubes, beakers, Solvent Chamber, Cappilary Tubes, Spraying Bottle etc.

Crude Drug:

The Plant *Datura stramonium* was collected from the area of Bani Malik of Abha city of asir Region of Saudi Arabia. It was authenticated by an experienced Pharmacognosist in Pharmacognosy, Department of College of Pharmacy, King Khalid University, Abha. The leaves of Plant were separated and shade dried at room Temperature. The drug was coarsely powdered with the help of Grinder.

Extract Preparation:

The Datura leaf powder was loaded in soxhlet apparatus. The apparatus was run with 100ml Absolute alcohol in attached round bottom flask by heating on water bath up to the siphoning tube showing no colored solvent. It was further run for 30 minutes to get if any transparent constituent get separated. The extract was collected and concentrated up to 20 ml of volume. This extract was kept for analysis.

TLC Fingerprint of Extract with Marker Compound (Qualitative Analysis):

The TLC Fingerprint of the extract was taken to see the presence of Atropine in the extract by using Standard atropine as Marker Compound. The Solvent system used was Hexane, Chloroform and Ethanol (20:50:30). The spraying Reagent used was Dragendorrf Reagent.

Quantitative Estimation of Atropine in *Daturastramonium* Leaf Extract

A- **UV-Estimation Method (New Method):**

Preparation of standard solution for calibration curve of Atropine

100mg of each Standard Atropine alkaloid was dissolved in 100ml solvent absolute Alcohol to produce a stock solution of 1000 µg/ml which was further diluted to produce the concentration of 0.01µg/ml, 0.02µg/ml, 0.03µg/ml, 0.04µg/ml, 0.05µg/ml, 0.06µg/ml, 0.07µg/ml, 0.08µg/ml, 0.09µg/ml, 0.10µg/ml. These Dilutions were kept for UV spectroscopic Analysis.

Determination of λ max: The dilute standard Atropine solution was scanned in the range of 200-

400nm to determine absorption maxima. The λmax was found to be 205nm.

Preparation of Calibration curve

The different dilutions for the standard Atropine were measured for absorbance at λmax 205nm. The calibration curve was plotted as concentration versus absorbance

Absorbance of Diluted Extract: The Extract was diluted to 100,000 times to get the concentration of Atropine (present in extract) in the range of concentrations of dilutions of Standard Atropine. The reported Value of Atropine in *Daturastramonium* is 0.2 to 0.6% (w/w).

B-Chemical method (Pharmacopoeial Method):

0.5 g of accurately weighed Atropine sulphate is dissolved in a mixture of 10 ml of glacial acetic acid and 10 ml of acetic anhydride. One to two drops of crystal violet IS is added and the solution titrated with perchloric acid (0.1 mol/L) VS until the color is changed to pure blue. A blank determination is performed and any necessary corrections made. Each ml of perchloric acid (0.1 mol/L) VS should be equivalent to 67.68 mg of Atropine Sulphate. From the experiment it was derived that 17 ml of perchloric acid was equivalent to 0.5 gmof Atropine alkaloid. A sample determination was done by using above method.

5. RESULTS AND DISCUSSION

Results

A) TLC Fingerprint of Extract with Marker Compound (Qualitative Analysis):

Figure 2: TLC Fingerprint of Extract with Marker Compound

- Pure Atropine Alkaloid as Marker Compound.
- First Spot (T)= Marker Compound
- Other Spots: Extract showing Atropine presence
- Rf Value of all spots= 0.08
- Solvent System: Hexane, Chloroform and Ethanol (20:50:30)
- The spraying Reagent used was Dragendorff Reagent.

B) UV spectroscopic Estimation of Atropine in *Daturastramonium*

(New Method for Atropine estimation in Plant Extract):

Determination of λ max: The dilute standard Atropine solution was scanned in the range of 200-400nm to determine absorption maxima. The λmax was found to be 205nm.

i) Absorbance(s) of Dilutions of Standard Atropine Solution:

Table 1:

Concentration (µg/ml)	Absorbance (at λmax 205nm)
0.01	0.030
0.02	0.061
0.03	0.090
0.04	0.120
0.05	0.153
0.06	0.180
0.07	0.211
0.08	0.241
0.09	0.270
0.10	0.305

ii) Absorbance of Diluted extract Sample:

Table 2:

Absorbance of Diluted extract Sample (at λmax 205nm)
0.090
0.091
0.089
Mean 0.090

Preparation of Calibration curve

The different dilutions for the standard Atropine were prepared across the range and absorbance was measured. The calibration curve was plotted as concentration versus absorbance.

B. Chemical Estimation of Atropine in *Daturastramonium* (Pharmacopoeial Method)

The 1 ml of the sample of the original extract was titrated with per chloric acid as per the standard protocol given in PRCC (Chinese Pharmacopoeia)

According to the standard protocol of PRCC and with the experiment, it was derived that 17 ml of perchloric acid is equivalent to 0.50gm of Atropine.

Therefore 0.1 ml of perchloric acid will be equivalent to 0.50gm×0.1/17=0.0029gm of atropine.

This is approximately 3mg of Atropine.

Calculation of concentration of Atropine in the leaves of the plant from above Methods:

The concentration of Original Extract was found to be 3mg/ml.

Since total Volume of the original extract was 20 ml.

The total Atropine in the sample was found to be (3mg/ml×20ml) =60mg

The crude drug (*Datura stramonium* Leaf Powder) taken for the extraction was 20gm.

Therefore 20gm drug contains 60 mg of Atropine.

100 % of drug will contain

60mg×100/20gm=6000mg/20gm%=6gm/20gm%=0.3 % (W/W)

After applying the calculations the concentration of Atropine was found to be 0.3% (w/w).

Discussion

TLC Fingerprint of Extract with Marker Compound (Qualitative Analysis Result) showing the presence of Atropine in the Datura extract. Since the both quantitative results were comparable and the values of the results were approximately equal supporting the validation of New Method (UV spectroscopic Method).

6. **CONCLUSION**

As we know that Atropine is the alkaloid (phytoconstituent) which is obtained from *Datura stramonium* (Solanaceae) andis of wide universal occurrence [3]. Geographical multiplicity is significant factor which have an effect on the active constituents of plants. [1,3] In spite of so many pharmacological importance's, up to the best of our knowledge till today atropine quantification by UV spectroscopic method in *Datura stramonium* is still not reported. Qualitative (TLC) Result showing the presence of Atropine in the Datura extract. The purpose of the study was to estimate the quantity of Atropine in *Datura stramonium*(leaf extract) of Saudi Arabian location. The two methods, UV spectroscopic Method and chemical method were employed to see the comparison in the results as well as to establish UV spectroscopic Method as the new method for quantitative estimation of Alkaloid. Hypothesis was made that two different analytical methods (UV spectroscopic method and Chemical method) are likely to yield comparable results. From the results and discussion, it was concluded that:

- The concentration of Atropine Alkaloid in the Saudi Arabian geographical variety of Datura stramonium was found to be 0.3% (w/w) which is close to the reported value.
- The new method for the quantitative estimation of Atropine i.e. UV spectroscopic method was developed successfully.
- The both results were comparable and the values of the results were approximately equal supporting the validation of New Method.
- The UV spectroscopic method can be employed in other similar studies of Alkaloid estimation in plant extracts.

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