**PHYSICOCHEMICAL PARAMETERS AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *DATURA METEL***

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**ABSTRACT-** Plants have great potential for the treatment and management of many diseases and have been used in many countries for the treatment of different diseased conditions. The medicinal value of plants lies in their bioactive phytochemical constituents that produce definite physiological actions in living beings. In modern era selection of drug and route of administration very much depends upon the chemical composition or alkaloids present in drug. *Datura* is a rich source of alkaloids such as Hyoscyamine, hyoscine, scopolamine, atropine, withanolides (lactones) and other tropanes. According to alkaloid present, the formulation containing *Datura* having antibacterial, antioxidant, herbicidal, antifungal, antiviral and antiulcer activity. Traditionally it is used in skin disorder, ear pain, cough, fever and asthma etc. Qualitative phytochemical test indicates the identification of primary metabolites (Carbohydrate, protein, fat etc) and secondary metabolites (alkaloids, glycosides tannin etc). **Aim-** Phytochemical & Physicochemical analysis through standardization of *Datura metel*. **Material & Method-** *Datura metel* was prepared and its physicochemical and phytochemical testing & standardization was done by following the standard protocols. **Results-** *Datura* was evaluated for different standardization parameters which showed Loss on drying (7.36%), Total ash(7.26%), Acid insoluble ash (3.65%), Water soluble extractive (17.65%), Alcohol soluble extractive (12.25%) and Water soluble ash (5.19%). Molisch test was positive in aqueous extract. Benedict test was positive in alcoholic extract. Fehling test was positive in aqueous extract. Alkaloids were identified in aqueous extract due to positive of Dragondroff test, Wagner’s test positive in aqueous extract and Hager’s test positive in aqueous extract. Amino acid was present in aqueous extract due to show positive result in Ninhydrine test. Proteins were present in test sample due to positive in Biuret test and Xenthoprotic test in aqueous extract. Foam test was positive in aqueous extract. Borntroger’s test positive in aqueous extract. Phenolic test was positive in aqueous and alcoholic extract. FeCl3 test, Pot. Dichromate test was positive in extract of seeds that’s indicate that tannin is present in sample. **Conclusion-** In *Ayurveda*, if every plant is used with *yukti*, it opens up new dimensions for humanity. Every plant is the gift of nature which can used for the well-being of mankind but this can only be done when we are well aware of its physicochemical and phytochemical properties. This paper highlights organoleptic characters and constituents of *Datura metel* and the data developed from the study can be espoused for allocating the standards for *Datura metel.*

**KEY WORDS-** *Datura,* antibacterial, antioxidant, herbicidal, antifungal, antiviral, antiulcer activity, standardization, physicochemical and phytochemical.

**INTRODUCTION-** *Datura* is known as a medicinal plant and plant hallucinogen all over the world. *Datura* has a very special place in *Ayurveda* since all parts of the plant namely leaves, flowers, seeds, roots, have been used for a wide range of medication such as treatment of leprosy, rabies, insanity, etc. The extract of *Datura*, however, is a potent poison and its indiscriminate use may lead to delirium and acute poisoning that may lead to death. The active constituents in *Datura* include scopolamine, atropine, hyoscyamine, withanolides (lactones) and other tropanes. Recently, withanolide compounds have shown significant antitumor, cytotoxic, anti-inflammatory, antibacterial, hepatoprotective, sedative, cytostatic and immunosuppressive activity. Due to increasing demand of herbal medicines by the population it is necessary to develop the pharmacopeial standards. To know the physicochemical characteristics and active principles, it is necessary to do analytical research. The drug before administered to human subject or experiment should be well understood & interpreted in the light of modern chemistry to know its proper scientific background. The purpose of the study is to find information regarding the drug to know its benefits and safety. Proper evaluation of the chemical composition helps in standardization of the drug.

**MATERIAL AND METHOD-**

**Collection of drug**

Fresh fruits were collected from herbal garden,shyampur,Nazibabad road, in the month of june. Fruits were dried in sunlight and seeds were collected for the study .

**Drug authentication**

The sample of raw drug was authenticated by expert of *Dravyaguna* department U.A.U. Rishikul campus, Haridwar.

**Standardization**

Standardization of *Datura* seeds was done and parameters were taken according to “Protocol for testing of *Ayurvedic Siddha* and *Unani* Medicines.” The sample was evaluated for organoleptic characters and for Standardization parameters like loss on drying, total ash, acid insoluble ash, Water soluble ash, water soluble extractive and alcohol soluble extractive.

**Loss on drying at 1050C[1]**

Moisture content is a water holding capacity of sample, higher moisture content in sample show that it may decrease stability.

Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105º for 5 hours. Calculated weight of sample for every 30 minute, until the weight of the sample came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.

**Calculation:**

Weight of the empty petridish = W1gm

Weight of the drug sample = X gm

Weight of the petridish with drug before drying (W3) = (W1 + X)

Weight of petridish after drying = W2gm

Loss on drying in % = W3-W2x100/X

**Determination of Total ash[2]**

Ash is a quantity analysis technique for determining siliceous material and inorganic substance in sample. Acid Insoluble Ash shows siliceous material and heavy metals. Water Soluble Ash shows quantity of water inorganic Substance.

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from the plant tissue itself and non-physiological ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to plant surface.

Silica Crucible was cleaned, dried well, labelled with glass pencils and then weighed to constant weight. 5 gm of powdered drug sample was put in the Silica crucible. The drug was spread evenly in to a thin layer. This crucible was placed in a muffle furnace and ignited at a temperature of 450°C for about 6 hrs or more until the ash was totally free from Carbon. The crucible containing the ash was allowed to be cooled in desiccators and subsequently weighed to constant weight. The percentage of ash with reference to the air dried drug was calculated.

**Calculation:**

Wt. of Empty Silica Crucible = A1gm

Wt. of Sample (X) = X gm

Wt. of the Crucible with Ash = A2gm

Percentage of Total Ash = [A2 - A1 /X] x 100

**Determination of Acid insoluble ash[3]**

Acid insoluble Ash value determined as per Pharmacopoeia of India, 1996. Boiled the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ashless filter paper. Washed with hot water, ignite, cool in a desiccator and weighed. Calculate the percentage of acid - insoluble ash with reference to the air - dried drug.

**Calculation:**

Wt. of drug sample - X gm

Wt. of Crucible = G1 gm

Wt. of Crucible with insoluble Ash = G2 gm

Wt. of insoluble ash (G3) = G2-G1

Percentage of acid insoluble ash = G3/X×100

**Determination of Water-soluble ash[4]**

Water – soluble ash value determined as per Pharmacopoeia of India 1996. Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch‘s Crucible or on an ash less filter paper, Washed with hot water and ignite for 15 minutes at a temperature not exceeding 450 C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water – soluble ash. Calculate the percentage of water – soluble ash with reference to the air - dried drug.

**Calculation:**

Wt. of drug sample - X gm

Wt. of total ash – A gm

Wt. of Crucible - G1 gm

Wt. of Crucible with insoluble Ash - G2 gm

Wt. of insoluble ash (G3) = G2-G1

Water soluble ash (G4) = Wt. of total ash gm- Wt. of insoluble(G3)

Percentage of water soluble ash = A – [(G3)/X] x 100

**Determination of Alcohol soluble extractive[5]**

5g. coarsely powdered air dried drug was macerated with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish was kept in oven at 105º, to constant weight and weigh. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

**Calculations:**

Weight of the drug material = X gm

Weight of the empty petri dish = W1gm

Weight of the perti dish with dried extract = W2gm

Percentage of extractive value =W2-W1x100/X

The procedure was repeated three times and the mean value was calculated.

**Determination of Water Soluble Extractive:**

Procedure was same as that of alcohol soluble extractive value and it was proceeded using distilled water instead of alcohol.

**Phytochemical screening[6]**

**Tests for Carbohydrates**

* Molisch’s test
* Benedict’s test
* Barfoed’s test
* Fehling solution test
* **Molisch’s Test:**
	+ 2ml of test Solution was taken in a test tube and 2 ml of the Molisch’s reagent was added and Shaken carefully and then about 1ml. of conc. H2SO4is poured from side of the test tube and allowed to stand for one 1 minute. A Purple colour ring at the junction of the two layers if formed indicated the presence of Carbohydrate.
* **Benedict’s test:**
	+ It is used for reducing sugars and composed of mainly Copper sulphate and sodium hydroxide. To the 4 ml of aqueous solution of drug, 1 ml of Benedict’s solution was added and heated almost to boiling. Formation of green, yellow, orange, red or brown colour in order of increasing concentrations of simple sugar in the test solution, due to formation of cuprous oxide.
* **Barfoed’s test :**
	+ The test sample was dissolved in water and heated with a little of the Barfoed’s reagent. Formation of red precipitate of cuprous oxide within two minutes indicates the presence of monosaccharides.
* **Fehling solution test:**
	+ It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium Potassium Tartarate.
	+ Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath. Formation of Brick red precipitate indicates the presence of reducing sugars.

**Tests for Alkaloids**

* Mayer’s reagent test
* Dragon Droff’s reagent test
* Wagner’s Test
* Hager’s Test
* **Mayer’s reagent test:**
	+ 2 ml of test Solution was taken in a test tube to which and 2 ml of the Mayer’s reagent (Potassium Mercury Iodide solution) was added. A White or Pale Yellow precipitate if formed indicated presence of Alkaloids except with Alkaloids of the Purine groups and few others.
* **Dragondroff’s reagent test:**
	+ 2ml of test Solution was taken in a test tube in which 2 ml of the Dragon Droff’s reagent (Mixture of Potassium Iodide and Bismuth sub nitrate solution) was added. An orange precipitate if formed indicated presence of Alkaloids.
* **Wagner’s Test:**
	+ Drug solution + few drops of Wagner’s reagent (dilute Iodine solution), formulation of reddish-brown precipitate.
* **Hager’s Test:**
	+ A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained which indicates the presence of alkaloids.

**Test for Amino acids**

* **Ninhydrin test :**
	+ The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules it gives characteristic deep blue or pale yellow colour due to the formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

**Tests for Proteins**

* Biuret test
* Xanthoprotic test
* Millon’s test
* **Biuret test :**
	+ A few mg of the residue was taken in water and 1 ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicates the presence of proteins.
* **Xanthoprotic test:**
	+ A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Development of yellow colour indicates the presence of proteins.
* **Millons test:**
	+ A small quantity of test sample was taken and 2 to 3 ml of millons reagent was added. The white precipitate slowly turning to pink, indicate the presence of proteins.

**Test for Saponin**

* **Foam test:**
	+ A small quantity of the test sample was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb like froth indicates the presence of saponins.

**Test for Glycosides**

* **Borntragor’s Test:**
	+ 1 ml of Benzene and 0.5 ml of dilute ammonia solution was added to the ethanolic extract and was observed for the formation of reddish pink colour.

**Test for Phenolic Compound**

* The extract was taken in water and warmed; to this 2 ml of ferric chloride solution was added and observed for the formation of green and blue colour.

**Test for Flavonoids**

* **Shinods test:**
	+ A small quantity of test sample was dissolved in 5 ml ethanol (95%v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5 gm of magnesium metal. Appearance of pink, crimson or magenta colour within a minute or two indicates the presence of flavonoids.

**Test for Steroids**

* **Salkoweski reaction :**
	+ Few mg of extract was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of test tube. The test tube was shaken for few minutes. The development of red colour indicates the presence of steroids.

**Test for Tannins**

* Ferric chloride solution
* Lead acetate
* Pot. Dichromate
* **Ferric chloride solution:**
	+ A 5 percent solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Appearance of dark green or deep blue colour indicates the presence of tannins.
* **Lead acetate :**
	+ A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. Development of precipitate indicates the presence of tannins.
* **Pot. Dichromate:**
	+ A solution of potassium dichromate was added to the filtrate. Appearance of dark colour indicates the presence of tannins.

**Chromatography:**

* **Chromatography plates-**

T.L.C. plate coated with 0.25 mm layer of silica gel 60 F254 with fluorescent indicator was used.  (Each plate dimension is 10 cm long and 2 cm width)

* **Activation of pre-coated Silica gel 60 F254 -**

Plates were dried in hot oven at 1050 C for one and half hour.

* **Test solution: Alcoholic Extract**
* **Preparation of mobile solution:** Toluene : Ethyl Acetate : Formic acid (6:3: 1)
* **Visualization:** Vanillin sulfuric acid Spray
* **Rf Value-**

Measured and recorded the distance of each spot from the point of its application and calculated Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

* **Calculation of Rf Value-**

                Distance travelled by solute from origin line

**Rf =**      ------------------------------------------------------------

                Distance travelled by solvent from origin line

**RESULTS**

Organoleptic characters, Physicochemical analysis, Phytochemical screening and Chromatography was done and results were summarised in Table (1), Table (2), Table (3).

**Table no. 1: Result of organoleptic characters of *Datura* seed *churna*-**

|  |  |  |
| --- | --- | --- |
| **S. No** |  **Organoleptic**  | **Observation**  |
| **1.** | **Colour** | Brown |
| **2.** | **Odour** | Odourless |
| **3.** | **Appearance** | Brown |

**Table no. 2: Results of Physicochemical characters of *Datura* seed *churna*-**

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No** | **Tests**  | **Value** | **Test method** |
| **1.** | **Loss on drying (%)** | 7.36 | **A.P.I, Part II, Vol-I,****Appendices- 2.2.10** |
| **2.** | **Aqueous Extractive Value (%)** | 17.65 | **A.P.I, Part II, Vol-I,****Appendices- 2.2.8** |
| **3.** | **Alcoholic Extractive Value (%)** | 12.25 | **A.P.I, Part II, Vol-I,****Appendices- 2.2.7** |
| **4.** | **Total Ash (%)** | 7.26 | **A.P.I, Part II, Vol-I,****Appendices- 2.2.3** |
| **5.** | **Acid Insoluble Ash (%)** | 3.65 | **A.P.I, Part II, Vol-I,****Appendices- 2.2.4** |
| **6.** | **Water Soluble Ash (%)** | 5.19 | **A.P.I, Part II, Vol-I,****Appendices- 2.2.5** |
| **7.** | **T.L.C.** | Rf Value : 0.27, 0.35, 0.41, 0.69, 0.74, 0.89, 0.94 |  **A.P.I** |

**Table no. 3: Results of Phytochemical Screening of *Datura* seed *churna*-**

|  |  |  |
| --- | --- | --- |
| **Name of Test** | **Aqueous Extract** | **Alcoholic Extract** |
| **Carbohydrate** |
| Molish test | **+ ve** | **+ ve** |
| Benedict test | **-ve** | **+ ve** |
| Fehling test | **+ ve** | **-ve** |
| **Alkaloids** |
| Dragendorff test | **+ ve** | **-ve** |
| Wagner’s test | **-ve** | **+ ve** |
| Hager’s test | **+ ve** | **-ve** |
| **Amino acids** |
| Ninhydrine | **+ ve** | **-ve** |
| **Protein** |
| Biuret test | **+ ve** | **-ve** |
| Xenthoprotic test | **+ ve** | **-ve** |
| **Saponin** |
| Foam test | **+ ve** | **-ve** |
| **Glycosides** |
| Borntrager’s test | **+ ve** | **-ve** |
| **Phenolic compound** |
| Phenolic test | **+ ve** | **+ ve** |
| **Steroids** |
| Salkowaski | **-ve** | **-ve** |
| **Tannins** |
| Fecl3 | **+ ve** | **-ve** |
| Lead acetate | **-ve** | **+ ve** |
| Pot. Dichromate | **+ ve** | **-ve** |

**Microbial contamination-**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No.** | **Microbial contamination** | **Value**  | **Reference Value** | **Test method** |
| **1.** | **Total bacterial count** | 102/g | **105/g** | **A.P.I, Part II, Vol-I, Appendices- 2.4** |
| **2.** | **Total fungal count** | 101/g | **103/g** |

**Aflatoxin**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No.** | **Aflatoxin** | **Value** | **Reference Value** | **Test method** |
| **1.** | **Aflatoxin B1** | Not Detected | **0.5 PPB** | **A.P.I, Part II, Vol-I, Appendices- 2.7** |
| **2.** | **Aflatoxin B2** | Not Detected | **0.1 PPB** |
| **3.** | **Aflatoxin G1** | Not Detected | **0.5 PPB** |
| **4.** | **Aflatoxin G2** | Not Detected | **0.1 PPB** |

**DISCUSSION**

The physicochemical analysis of *Datura* seeds done through the standard protocol mentioned in *Ayurvedic* Pharmacopeia of India. Organoleptic study was done by using the sense organs. During the organoleptic study appearance of *Datura* seeds were coarse powder, Brown in color and odourless in odour. Ash value is the common method to know the adulteration of the inorganic materials, and it has greater importance in the quality control and standardization. Total ash value of *Datura* seeds in this study shows lesser amount of inorganic material. Acid insoluble ash represents presence of silica and silicate impurities. *Datura* seeds contains 3.65% of siliceous content. Extractive values indicated the presence of different constituents and TLC fingerprint can be developed for identification and semi quantitative analysis from these extracts. Water soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying on storage or formulating. Water soluble extractive value of *Datura* seeds is 17.65 %. The alcohol soluble extractive value is also indicative for the same purpose as the water soluble extractive value, which is showing *Datura* seeds have 12.25 % extractive value. Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. This study is done in alcoholic and aqueous extract, Qualitative analysis for the presence of various functional group revealed the presence of Carbohydrate, Alkaloids, Amino acids, Protein, Saponin, Glycosides, Phenolic compounds, Steroids and Tannins. At high levels, aflatoxins can cause illness and liver damage. *Datura* seeds are free from aflatoxins.

**CONCLUSION**

The present study gives very imaginative information related to its Physicochemical and phytochemical analysis which will be helpful for standardization of *Datura* seeds. It opens doors to the researchers to do more and more research in the field of *Ayurveda* to explore new things, to find out cost effective cure for various diseases.

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