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PHYTOCHEMICAL ANALYSIS OF ASHWAGANDHA

Rajeev Kushwah[1],M C Sharma[2], Nirmala Kushwah [3], Deepa Sharma [4]Pawan vishwakarma [5]

[1]Lecturer Deptt. of Dravyaguna Bundelkhand Govt. Ayurveda College & Hospital Jhansi, Uttar Pradesh, India

[2] Director SBLD Vishwa Bharti, Sardar Sahar Former Director National Institute of Ayurveda, Jaipur, Rajasthan, India

[3] Medical Officer, Ayush Wing Tikamgarh, Madhya Pradesh, India

[4] Lecture,Deptt. of Rog Nidan & Vikriti Vigyan Major S.D.Singh Ayurvedic Medical College,Fatehgarh Farukhabad, Uttar Pradesh, India

[5]Lecturer Deptt. of Bal Rog Bundelkhand Govt. Ayurveda College & Hospital Atarra, Uttar Pradesh, India

ABSTRACT

Ayurveda cannot remain confined to the use of conventional, conservative norms of medication. It has to accept the new challenges and be preparing to answer the queries of the modern man who would have a right to know about the drug he is consuming. To meet this new thrust of inquisitiveness, standardization of drugs of Indian systems of medicine is mandatory. Chemical analysis of any drug should be known well before experimental and clinical trials. The first scientific investigation on the chemical principles of this plant appeared in the year 1911, when Power and Salway reported the presence of a number of compounds from the roots and leaves of Withania somnifera, namely, two new monohydric alcohols, withaniol, C_5 H_{33} O_4 OH and somnirol C_{32} H_{43} O_6 OH. In this section I have done analytic study of *ashwagandha.*

Key word*: ashwagandha*, analytic study, TLC , spectrophotometry

INTRODUCTION

Phytochemistry is the branch of natural product chemistry in which qualitative and quantita-tive investigation of herbal drugs take place. Phytochemistry is in the strict sense of the word, the study of phytochemicals. These chemicals are derived from plants. In a narrower sense, the terms are often used to describe the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attacks and plant diseases. They also exhibit a number of protective functions for human consumers. The systematic investigations of plant materials for its phytochemical behavior involves for different stages.

- The procurement of drug material and its quality control.
- Examination, Purification and Characterization of the constituents for pharmaceutical interest and in process of quality control.
- Investigation of Bio-synthetic pathways of a particular compound.
- Quantitative evaluations.

Standardization is starts right from the collection of raw materials to the extreme clinical application. In case of Ayurvedic medicines, the therapeutic efficacy is a total effect of its chemical consti-tuents. So, the quality and purity refers to the total profile of the drug rather than any of its charac-ter. Therefore, a multidimensional approach is essential for standardization of Ayurvedic drug. This multidimensional approach should cover every minute aspect of Ayurvedic drug specifically the name, botanical source, geographical source, organo-leptic, morphological, anatomical, physical, chemical and biological activities.

Phytochemical Examination:

In the plants, two types of phytochemicals are present,

Inorganic matters

Organic matters.

Inorganic Matters are those which are free from carbon i.e. all electrolytes comes under inorganic matter. Organic Matters are secondary metabolite products in the plants. Role of these components for medicinal purposes are important. Examinations of both Organic and Inorganic matters were done both qualitatively and quantitatively.

Qualitative examination of Inorganic matters:

It involves qualitative examinations of electrolytes, which are present in ash of the sample. Higher plants, for growth and reproduction require sixteen or Seventeen elements. Elements re-quired in relatively large quantities are termed as macronutrients, where as nutrients required in small amounts are termed as micronutrients. All such elements are used up in various metabolic processes. Variety of organic compounds synthesized by the plants incorporates these elements in their chemical constitutions. Therefore, their presence can be detected by simple chemical analysis.

Aims & Objects:

The phytochemical analysis of Ashwagandha curna was carried out for regulating the au-thenticity of the test drug as well as to determine the various constituents.

MATERIAL & METHODS:

The drug i.e. Ashwagandha was pharmacognostically identified. The dried sample was powdered and then used for the present study. The analysis of sample was carried out with a view to evolve suitable parameters for evaluating their quality and also to analyze the data. The phytochemical analysis of drug was carried out in the Pharmaceutical Laboratory of National Institute of Ayurveda, Jaipur. The study was carried out according to following plan of study like determination of various constituents-

Drug Sample: ASHWAGANDHA

Preparation of Test Sample

5 gm of drug samples were taken in crucibles. The crucibles were kept in muffle furnace at 550□C for nearly 6 hours. After 6 hours, the ash was removed from the muffle furnace. The ash was dissolve in 5ml of slightly acidic water and this solution was used for detecting the presence of mineral elements.

Calcium: 0.5ml of test sample and 2 drops of Conc.H2SO4 was taken. White ppt was formed. it was indication of the presence of calcium.

- Iron: 0.5 ml of test sample and 3 drops KSCN reagent was taken. Formation of red colour indicate the presence of Iron.
- Manganese: 0.5 ml of test solution was taken and was added 1ml of 1% KOH solution then 5 drops of Benzedine reagent. Formation of blue colour showed presence of Mn.
- Phosphorus: 0.5 ml of test solution was taken and two drops of Ammonium Molybdate reagent was added. Formation of yellow colour indicate the presence of Phosphorus.
- Potassium: 0.5 ml of test solution was taken and 2 drops of 15% HClO4 soln. Formation of KClO4 crystals indicate the presence of K.
- Sulphur: 0.5 ml of test solution was taken and 2 drops of BaCl2 was added.Formation of white ppt. of BaSO4 indicate the presence of Sulphur.

Qualitative Analysis of the Ashwagandha is as follows:

Table No- 2

S. No	Parts of the Drug	Name of the Minerals / Electrolytes					
		Calcium	**Iron**	**Manganese**	**Phosphorus**	**Potassium**	**Sulphur**
1.	Root	+	+	+	+	+	+

Determination of Heavy Metals:-

COBALT: Dissolved 20 mg of the ash of the drug in about 0.5 ml of distilled water, and acidified with a few drops of dip hydrochloric acid. A few drops of dilute solution of sodium hydroxide were added. No blue pit was formed which indicate the absent of cobalt..

COPPER: Dissolved 20 to 25 mg of the drug in 1 ml of distilled water and dilute ammonia solution was added, drop wise until a clear blue solution

was obtained. Heated to boiling and added drop wise 2% W/V alcoholic solution of α-benzoinoxime. No green ppt was formed which indicate the absent of copper.

MERCURY: Dissolved 20 to 25 mg of the ash of the drug in 1 ml of distilled water, and 2 M sodium hydroxide was added until solution became strongly alkaline. No dense yellow ppt was formed which indicate the absent of mercury.

NICKEL: 20 mg of the ash of the drug was added in about 0.5 ml of water, acidified with a few drops of dilute hydrochloric acid, and then solution of sodium hydroxide was added drop by drop. No blue ppt was formed which indicate the absence of nickel.

SILVER: 20 to 25 mg of the drug in 2-3 ml of distilled water was added and then 0.2 ml of 7 M hydrochloric acid was added. A curdy white ppt was formed that is soluble in 3 ml of 6 M ammonia. A few drops of a 10% W/V aq sol. of potassium iodide were added. No yellow ppt was devel-oped. It indicates the absence of silver.

ZINC: Dissolved 20 to 25 mg of the ash in 2 to 3 ml of distilled water, and 0.2 ml of 10 M sodium hydroxide was added. A white ppt was formed which dissolved in 2 ml of 10 M sodium hydroxide solution. About 5 ml of 2 M ammonium chloride was added followed by 0.1 ml of sodium sulphide solution. A flocculent, white ppt was produced. It indicates the presence of zinc.

Table No- 3 Qualitative Analysis of the Ashwagandha is as follows:

S. No	Parts of the Drug	Name of the Heavy Metal					
		Cobalt	**Copper**	**Mercury**	**Nickel**	**Zinc**	**Silver**
1.	Root	-	-	-	-	+	-

Quantitative Examination of Inorganic Matter:

The quantitative Examination include the following examinations

Determination of foreign matter

Apparatus: Physical balance, paper sheet, bull lens.

Method: The electronic monopan balance weighed 100 gram of sample () and a thin layer of sample was spreaded on a white colour sheet. By bull lens, the layer was examined for foreign matter. No foreign matter was seen. The drug sample was recollected and weight again. Weight of sample was 100gm. Therefore we can say foreign matter was absent in my sample.

Result No foreign matter was seen in the sample.

Standard not more than 2%

Remark Foreign matter may be moulds, insects, animal excretory matter and other contaminations like stone & extraneous material.

References API /APPENDIX II / PAGE NO 142 / 2.2.2.

Determination of pH

Apparatus: beaker, conductivity meter, rotator shaker, pH meter, distilled water, physical balance

Method

1. Conductivity of distilled water was checked by conductivity meter. It was found to be 10 (conductivity 10 or less than 10 is required)
2. By electronic monopan balance 10 gm of sample was weighted in a beaker.
3. This sample was dissolved in 50 ml of distilled water.
4. The beaker was kept in orbitek rotatory shaker for shaking for one hour.
5. Then the beaker was put under previously calibrated electrode of the pH meter.

6 pH meters gave reading 5.33

Standard not more than 2%

Result pH of the sample was 5.33.

References API /APPENDIX III / PAGE NO 156 / 3.3

Determination of Moisture Content

Apparatus

Beaker, hot air oven, desiccators, paper sheet, physical balance

Method

- Empty beaker was kept in hot air oven for ½ hr and then in desiccator for ½ hr.
- Therefore, that it became moisture free.
- Initial wt. of beaker was noted.

- 10 gm drug powder was taken in three beakers for 3 individual readings.
- Beakers were kept in hot air oven at 1050 C for 5 hours.
- Then the beaker was taken out and kept in desiccator for ½ hr.
- Wt. of beaker was taken and noted.
- Then again, beakers were kept in oven and then in desiccator till the wt. become constant.
- Final weight of the beakers were noted.

Weight of the Empty beaker = W1 gm
Weight of the Drug Sample = X gm
Wt. of the beaker with drug before drying = W3= (W1+X)
Weight of beaker after Drying = W2 gm
Loss on Drying in % = $\frac{W3 - W2}{X} \times 100$

Table No- 4: Calculation for determination of Loss on drying of Ashwagandha: Root

Sr. no	Intial wt. of beaker W_1	Initial wt. of drug X	Final wt. of beaker before drying W_3 (W_1+X)	Final wt. of beaker with drug after drying W_2	Loss on drying in gm.	Moisture content in %
1	66.477 gm	10 gm	76.477 gm	75.876	0.601	**6.01%**
2	61.283 gm	10 gm	71.283 gm	70.688	0.595	**5.95%**
3	**63.750 gm**	**10 gm**	**73.750gm**	**73.102**	**0.648**	**6.48%**

Average Moisture content in % = 6.147 %

Determination of Total Ash

Three Silica Crucibles were cleaned, dried well and then weighed to constant weight and labeling was made A1, B1, and C1. 3gm and 5gm of the drug sample were weighed accurately and placed in the Silica Crucibles respectively. These crucibles were placed in a muffle furnace at a temperature of 450□C □5□C till were become totally free from Carbon. The time spends for this process was about 6 hrs. The crucibles containing the ash were allowed to be cooled in desiccators and subsequently weighed to constant weight.

Calculation
Wt. of Empty Silica Crucible = A1, B1, C1 gm
Wt. of Sample (X) = X gm
Wt. of the Crucible with Ash = A2, B2, C2 gm
Percentage of Total Ash = $\frac{A2 - A1}{X} \times 100$

The process was repeated three times for each drug sample and the Average Total Ash value was calculated.

Table No- 5 :Observations for determination of total ash of Ashwagandha: Root

S. No	Weight of the sample (X)	Weight of empty crucible (A_1)	Weight of Crucible with ash (A_2)	Percentage of total ash $[(A_2-A_1)/X] \times 100$
1.	5 gm	44.7265gm	44.9658gm	4.786%
2.	5 gm	32.5042gm	32.7413gm	4.742%
3.	5 gm	29.9730gm	30.2095gm	4.73%

Result - The Average Percentage of Total ash =4.75%

Standard not more than 7%

References API /APPENDIX II / PAGE NO 143 /2.2.3

Determination of Acid Insoluble Ash

The ash obtained from above procedure was added to 100 ml of diluted HCl (2N) in a beak-er. Then the mixture was heated at temperature of 70 to 80□C for 5 minutes. The mixture was fil-tered into a pre-weighed Gooch's Crucible fitted with a Whatman's filter paper No 1, transfixed to a beaker that was attached to vacuum pump. The glass beaker containing Ash and HCl mixture was then washed with boiling water three times and the water was also poured to the Gooch's Crucible.

This mixture was allowed to be filtered by using the vacuum pump. The Gooch's Crucible with re-sidual Ash was then dried in the oven at 50□C till completely dried. Then it was allowed to be cooled in desiccators and subsequently weighed and the Acid Insoluble Ash was calculated.

Calculation

Wt. of Drug sample = X gm

Wt. of Empty Gooch's Crucible with filter paper = G1gm

Wt. of the Gooch's Crucible with residual Ash = G2 gm

Percentage of Acid Insoluble Ash = [G2- G1/X]×100

The procedure was repeated three times for each sample and the average value was calculated.

Table No- 6 Observations for determination of Acid insoluble ash of Ashwagandha:

□ **Root**

S. No	Weight of the sample (X)	Weight of the filter paper (G_1)	Weight of filter paper with Residual ash (G_2)	Percentage of acid insoluble ash $[(G_2-G_1)/X] \times 100$
1.	5 gm	0.8216gm	0.8471gm	.51%
2.	5 gm	0.8196gm	0.8448gm	.50%

Result

The average percentage of acid insoluble ash = 0.505%.

Standard not more than 1%

References API /APPENDIX II / PAGE NO 143 /2.2.4

Determination of Water Soluble Ash: -

The Ash obtained from above procedure (Ash of 3/5 gm drug) was mixed with 100 ml of distilled Water in a beaker and the mixture was heated at 70-80□C for 5 minutes. This mixture was poured into a pre-weighed Gooch's Crucible fitted with a Whatman's filter paper transfixed in a beaker attached to a vacuum pump. The mixture was filtered into the beaker. The Gooch's Crucible with residual insoluble Ash was dried at 50□C in oven and then allowed to be cooled in desiccator and subsequently weighed and the Water Soluble Ash was calculated.

Table No- 7 **Calculation**

WT. OF THE EMPTY GOOCH'S CRUCIBLE WITH FILTER PAPER = G_1 GM
Wt. of Drug Sample = X gm
Wt. of the Gooch's Crucible with Water Insoluble Ash = G_2 gm

Wt. of Total Ash	= A gm
Percentage of Water Soluble Ash	$=A-[(G_2- G_1)/X] \times 100$

The procedure was repeated three times for each sample and average value was calculated.

Table No- 8 Observations for determination of Water soluble ash of Ashwagandha:

 Root

S. No	Wt. of the sample (X)	Wt. of the Total ash (A)	Wt. of empty filter paper (G_1)	Wt. of filter paper with water insoluble ash (G_2)	Percentage of water soluble ash $\{[A-(G_2-G_1)]/X\} \times 100$
1.	5 gm	.3393gm	.9157gm	1.2026gm	.974%
2.	5 gm	.3371gm	.9086gm	1.1968gm	.978%

Result -The average percentage of water soluble ash = .976%

Standard not more than 1%

References API /APPENDIX I / PAGE NO 143 /2.2.7

Qualitative Examination of Organic matter:

Study of all organic active compounds in medicinal plant was done as follows -

(1) Carbohydrates

The dry weight of plant, typically composed of 50-80% of the polymeric Carbohydrate Cellulose along with related structural material. Carbohydrates are the backbones of Nucleic Acids. These are synthesized in plants by photosynthesis from CO2 and H2O. The fundamental principles of life are catalyzed by the green plant pigment, Chlorophyll and enzyme system and liberate oxygen.

n CO2 + n H2O (CH2O) n + n O2

The name Carbohydrate arises from Hydrates of Carbon, since the molecular formula of many could be expressed in the form of Cx (H2O) y.

Qualitative Tests

- Molich's Test - 2 ml of the Aqueous Extract of Drug was taken in test tube and 2 ml of the Molisch's reagent was added and shacked carefully, then about 1 ml. of conc. H2SO4 was poured from side of the test tube and allowed to stand for one 1 minutes. A red brown ring was appeared at the junction of the two layers indicated the presence of Carbohydrate.
- Detection of Starch - 2 ml of Iodine soln. was taken in a test tube and then 2 ml aq. soln. of Drug was added to it. Appearance of Blue- Black colour indicated the presence of Starch.

(2) Alkaloids

These are derived from 'Alkali' (alk=alkali, oid = like). They may be defined as Organic Nitrogen-ous substances of plant origin exhibiting well defined physiological action. Alkaloids exhibit a va-riety of physical and chemical properties. The free alkaloids are insoluble in water but their salts are freely soluble. These are mainly three types:-

- True alkaloids: Those having a Heterocyclic ring with Nitrogen atom.
- Proto alkaloids: They don't have Heterocyclic ring with Nitrogen atom.
- Pseudo alkaloids: They have Heterocyclic ring with Nitrogen atom but not derived from Amino acid. 1&2 both are derived from Amino acids.

Qualitative Test: The following colour tests are used to detect the presence of an Alkaloid in a Drug sample.

- Mayer's reagent - It is Potassium Mercury Iodide soln. & gives a White or Pale Yellow ppt., except with Alkaloids of the Purine groups and few others.
- Dragon Droff's reagent - It is soln. of Potassium Iodide and Bismuth sub Nitrate. They form Orange colour ppt. with the reagent.

(3) Proteins

Proteins are highly complex molecules, which contain the elements of Carbon, Hydrogen, Nitrogen and occasionally Sulphur. They are synthesized by living cells and are essential part of the structure of the cell and its nucleus. The plant proteins are more easily isolated in crystalline form.

Proteins are stored in plants in the form of aleurone grains and are required for animals as the source of nitrogenous food. Proteins are hydrolysed to form simpler substance i.e. Amino acid.

Protein → Polypeptide → Peptid → Amino acid

Qualitative Test

Ninhydrin Test - To an Aqu. Soln. of Protein, Alc. soln. of Ninhydrinwas added and then heated. Formation of Red/Blue to Violet colour indicated the presence of Proteins.

(4) Tannins: Tannins are derivative of Benzoic acid, which are widely distributed in the Vegetable Kingdom.

Tannin precipitates and combines with Proteins and the Protein-Tannin complex is resistant to proteolytic enzymes. This property is known as Astringent. During heating process of Burns, the Proteins of the exposed tissue are ppt. producing a mildly Anticeptic, under which the new tissues are regenerated. Tannins are used in the tanning process of animal hides to convert them into Leath-er.Aqu.soln of Tannins are used to ppt. Gelatine, Proteins and Alkaloids in the laboratory. They are used as healing agent, in Inflammation, Leucorrhoea, Gonorrhoea, Burns, Piles, Diarrhoea and as Antidote in the treatment of Alkaloidal poisoning .The deep Red coloured complex with Iron and salt used to manufacture Inks.

Test for Tannin: When Aqu. Extract of the drug was treated with Vanillin HCl Alcohol reagent (Vanillin 1gm + 10 ml conc. HCl +10 ml Alcohol), Brick or Red colour was formed, showing the presence of tannin.

(5) Glycoside : Glycosides are compounds, which upon hydrolysis give rise to one or more Sugars (Glycones) and a compound, which is not a Sugar (Aglycone or Genin). Glycosides are use was added.d as Cardiac stimulant; Laxative, Bitter Tonics, Hepatoprotective and some other have Ex-pectorant properties.

Qualitative Test: Killer – Killiani Test - To an Extract of drug in Glacial Acetic Acid, a few drops of FeCl3 and conc. H2SO4 were added. Formation of Reddish Brown colour at the junction of two layers and changing of the upper layer into Bluish Green indicates presence of Glycoside.

Legal Test - To a soln. of Glycoside in Pyridine Sodium Nitroprusside solution, add NaOH soln. Pink Red colour formation indicates presence of Glycoside.

(6) Sapponin: Saponins are highly complex Glycosides, which are widely distributed, in the higher plants. Saponin forms colloidal soln. in water, which gives soap like froth on shaking. They have property of causing haemolysis of Red Blood Corpuscles (RBCs) even at great dilution; most of the Saponins are highly toxic when injected into the body. So it should be taken orally. They yield an Aglycone known as Saponin on hydrolysis, which made Steroid or Triterpene and Sugar moiety, which may be Glucose, Galactose etc. Saponins are related with compounds such as Sex hormones, Cortisone, Diuretic, Steroids and vitamin D.

About 1 ml of Aqueous Extract was diluted by distilled Water up to 10 ml and shaked in a graduated cylinder for 15 minutes. No Formation of froth indicated the absence of Saponin.

(7) Phenols:These are Alcoholic derivatives of Benzene. These are secondary metabolic products. They have hazardous property for all living organism and they combine with Sugars and form Phe-nolic Glycosides.

Qualitative Test for Phenols

2 ml of Drug extract was taken in a test tube and added 2 ml of FeCl3 solution. Blue – Violet/ Red or Deep Green colour of the solution was indicate to presence of Phenols.

Table No- 9 **Observation of Qualitative analysis of Organic matter in drug Ashwagandha**

S. No.	Chemical constituent	Test Applied	Result (Root)
1.	Carbohydrates	Molisch's reagent	+
2.	Starch	Iodine solution	+

3.	Tannin	Vanillin solution	+
4.	Protein	Ninhydrin solution	+
5.	Saponin	Shaking with water	-
6.	Phenol	$FeCl_3$ solution	+
7.	Glycoside	Killer Killiani test	+
8.	Alkaloid	Dragondroff's reagent	+

Quantitative Examination for Organic matter:

Determination of Extractive values:

The organic substances of the different parts of Ashwagandha show their solubility in various, solvents in different quantities. So for this purpose of determination of extractive values seven main solvents were selected according to their polarity.

1. Chloroform
2. Ethyl acetate
3. Acetone
4. Methanol
5. Water.

Coarsely powdered air dried drug material is accurately weighed and taken in a glass stopper conical flask. Solvent is added to the flask and the flask is attached to a reflux condenser and boiled for 2hrs, on water bath. After 2hrs, the flask is allowed to cool and the content is filtered through filter paper. The filtrate is transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish is kept in oven for six hours for the contents to get dried fully. The Dish is cooled by keeping in a desiccator for 30 minutes and weighed without delay.

The residual mass remained in filter paper is dried as such and is collected fully. This mass in again put into the conical flask and added with next solvent according to polarity, and fitted with reflux condenser, and extract is prepared in the same method used above. This procedure is repeated with all the seven solvents. The content of the extractable matter is calculated in the following man-ner.

Calculations:

Weight of the drug material = Xgm

Weight of the empty petridish = W1gm

Weight of the pertidish with dried extract = W2gm

Percentage of extractive value $= \frac{(W2-W1) \square 100}{X}$

The procedure was carried out with same drug sample with different solvent taken in the or-der of polarity.

Table No- 10 **Determination of Extractive values of Ashwagandha Root**

S. No	Solvent	Weight of the drug (X)	Weight of Empty Petri dish (W_1)	Weight of Petridish+Dried extract(W_2)	Percentage of Extract $(W_2-W_1)/X \times 100$
1.	Chloroform	100gm	33.2630gm	33.7993 gm	**0.5363%**
2.	Ethyl acetate	100gm	35.4520gm	35.8554 gm	**0.4034%**
3.	Acetone	100gm	34.8875gm	35.7658 gm	**0.8783%**
4.	Methanol	100gm	35.4636gm	37.8695 gm	**2.4059%**
5.	**Water**	**100gm**	**34.5172gm**	**42.9844gm**	**8.4672%**

Total alkaloid Apparatus: Flask, vacuum dessicator, sulphuric acid, dragondraf's Reagent, ace-tone, hydrogen sulphide, ethyl alcohol.

Method: Take about 30 gm accurately weight of the powdered drug, cover with alcohol

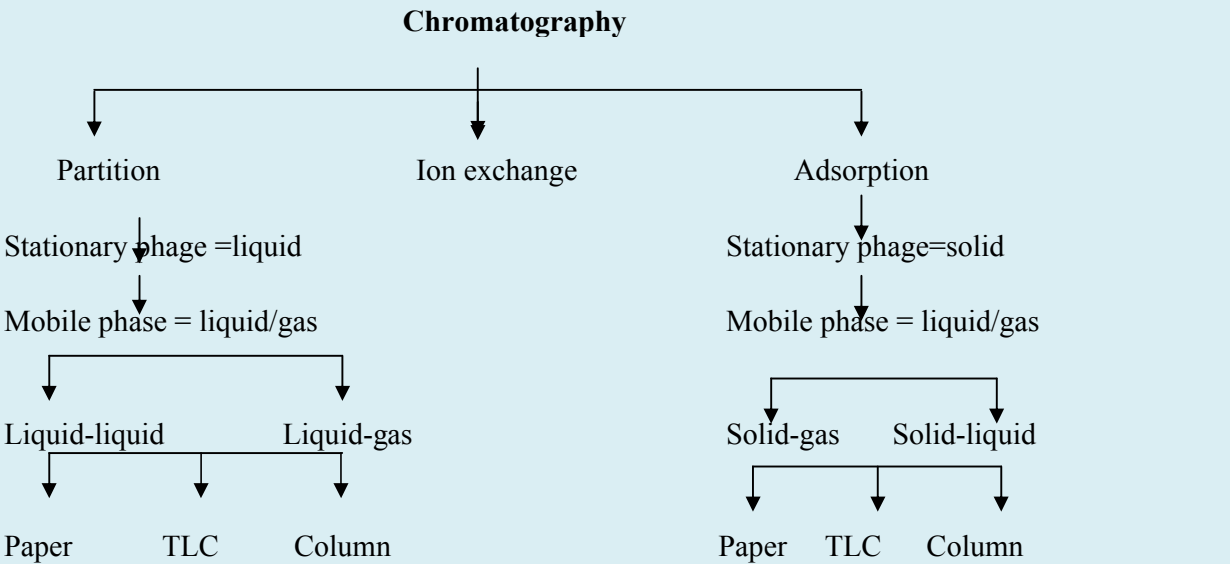
- (90 %) and allow to stand overnight.
- Extract for 6 hr.so wet apparatus and concentrate to a syrup residue
- Treat with 25, 20, 15 and 10 ml portion of 5% sulphuric acid until complete extraction of alkaloid was affected.

- To the combined acid extracts add an excess of dragandorf's reagent.
- Filter under suction and dissolve the residue in acetone, shake the acetone solution with freshly prepared suspension of 2 gm silver carbonate in 10 ml of water.
- Filter the solution and wash the precipitate with acetone, alcohol and water in that order.
- Pass sufficient hydrogen sulphide through the filtrate. Boil the solution for 10 min., filter and evaporate under vacuum in a tared flask.
- Added to the residue 5 ml of ethyl alcohol, evaporate to dryness, repeat the process once again and weight the residue to constant weight in a vacuum dessicator.

- **Calculation**

Wt. of empty flask	A	57.2590
Wt of flask with residue	B	57.3348
Wt of residue	C	B-A
		57.3348-57.259 = 0.0858 gm

- In 30 gm wt of total alkaloid is 0.0858 gm
- In 1 gm wt of total alkaloid is 0.0858 / 30 = 0.00286 gm
- In 100 gm wt of total alkaloid is 0.00286 * 100 = 0.286
- % of total alkaloid 0.286 %
- Result = % of total alkaloid 0.286 %
- **CHROMATOGRAPHY**
- Chromatography
- Chroma = colour, Graphy = writing
- Chromatography is the collective term for a family of laboratory techniques for the separa-tion of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyze to be measured from other molecules in the mixture and allows it to be isolated. Essentially, the technique of chromatography is based on the difference in the rate at which the component of a mixture move through a porous medium (called stationary phase) under the influence of some solvent or gas (called moving phase)
- Classification of chromatography
- The moving phase may be a liquid or a gas. Based on the nature of the fixed and moving phase different type of chromatography are follows:-



Adsorption chromatography

It is based on the differences in the adsorption coefficients. In this the fixed phase is a solid e.g., Alumina, Magnesium oxides, Silica gel etc. The solute is adsorbed in different part of the adsorbent column. The adsorbed components are then eluted by passing suitable (solvent) through the column.

Partition chromatography

It operates by mechanism analogous to counter-current distribution. The fixed phase may be a liquid strongly adsorbed on a solid which acts as a support. In this case, the solute gets distributed between the fixed liquid and the moving liquid (solvent). The technique is called partition chromatography.

THIN LAYER CHROMATOGRAPHY (T.L.C.)

Thin layer chromatography is a technique to separate the compounds from a mixture based on adsorption principle. It has the advantage of faster runs, better separations, and the choice be-tween different adsorbents. Different compounds in the sample mixture travel different distances according to how strongly they interact with the adsorbent. This allows the calculation of an Rf val-ue and can be compared to standard compounds to aid in the identification of an unknown sub-stance.

Calculation of Rf Value

- Distance traveled by solute from origin line
- Rf = ----------
- Distance traveled by solvent from origin line

- Chromatography plates-
- T.L.C. plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator, (Mercks) were used. Each plate having dimension 10 cm long and 1 cm width.
- Detection – Long wave and short wave of U.V. radiation. Iodine vapour
- Solvent System- According to their increasing order of polarity.
- T.L.C. of Ashwagandha

Acetone Extract

Table No- 11 **Mobile phase- Dichloromethane: Methanol:: 9.5:.5**

Spot No.	Distance traveled by solvent	Distance traveled by solute	Rf Value
1	6.5	0.5	0.0769
1.	6.5	1.0	0.1538
2.	6.5	1.4	0.2153
3.	6.5	1.8	0.2769
4.	6.5	2.5	0.3846
5.	6.5	3.0	0.4615
6.	6.5	4.0	0.6153
7.	6.5	4.9	0.7538
8.	6.5	5.6	0.8615

Chloroform Extract

Mobile phase Toluene: Chloroform:: 1:9

Table No- 12 **Solvent run: 4.1cm Visualization:**

Spot No.	Distance traveled by solvent	Distance traveled by solute	Rf Value
1.	4	0.6	0.15
2.	4	1	0.25
3.	4	2	0.5
4.	4	2.4	0.6
5.	4	3	0.75
6.	4	3.4	0.85

7.	4	3.9	0.975

Table No- 13 **Methanol Extract Mobile phase-Solvent Chloroform: Methanol::8:2 Solvent run:7cm**

Spot No.	Distance traveled by solvent	Distance traveled by solute	Rf Value
1.	7	1.5	0.214
2.	7	2	0.285
3.	7	2.8	0.4
4.	7	3.2	0.457
5.	7	3.6	0.514
6	7	4.5	0.642
7	7	5	0.714
8	7	5.8	0.828

U.V. Sphectrophotometry

In physics, spectrophotometry (EC Doublebeam UV-VIS sphectrophotometer UV2540SS) is the quantifiable study of electromagnetic spectra. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared. Also, the term does not cover time-resolved spectroscopic techniques.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light. There are many kinds of spectrophotometers. Among the most important distinctions used to classify them are the wavelengths they work with, the mea-surement techniques they use, how they acquire a spectrum, and the sources of intensity variation they are designed to measure. Other important features of spectrophotometers include the spectral bandwidth and linear range.

Perhaps the most common application of spectrophotometers is the measurement of light absorption, but they can be designed to measure diffuse or specular reflectance. Strictly, even the emission half of a luminescence instrument is a kind of spectrophotometer..

The use of spectrophotometers is not limited to studies in physics. They are also commonly used in other scientific fields such as chemistry, biochemistry, and molecular biology They are widely used in many industries including printing and forensic examination

U.V.Sphectrophotometric Analysis

When chloroform extract (0.5 mg/ml) was used, maximum wavelength (□max) was observed at 241.0 nm with 2.453 absorbance when it was scanned in range 220.00-550 nm we observed the values, 241.0 nm with 2.466 absorbance as shown in. When chloroform extract i.e. 1 mg/ml, We observed the maximum wavelength 272.5 nm with absorbance 2.380 . These results were according to the principle of UV vis spectrophotometer.

We observed maximum wavelength (□max) at 333.5 nm with 0.760 absorbance for acetone extract (0.5 mg/ml) , (□max) at 336 nm with 1.454 absorbance When acetone extract (1 mg/ml) was used.

When methanol extract (1mg/ml) was used, maximum wavelength (□max) was observed at 242.5 nm with 2.497 absorbance. further dilution of the methanol extract (0.50 mg/ml), maximum wavelength was the 237.5 nm with absorbance 2.499.

DISCUSSION

The drug used for clinical trial was subjected to Physiochemical analysis prior to its administration. The observation made during the Physiochemical analysis of Ashwagandha Churna ac-cording to different parameters are discussed below:

All the result perceived was in accordance with the standards laid down in Ayurvedic Phar-macoepia of India. Vol. I 2002.

Table 14 : Analytical Study of Ashwagandha

S.No	*Investigation*	*Ashwagandha*	*API Standard*

1.	Foreign Matter	Nil	Not more then 2 %
2.	pH	5.33	Not mention
3.	Moisture Content	6.147 %	Not mention
4.	Total ash	4.75%	Not more then 7 %
5.	Acid insoluble ash	0.505%.	Not more then 1 %
6.	Water soluble ash	.976%	Not mention
7.	Chloroform	0.5363%	Not mention
8.	Ethyl acetate	0.4034%	Not mention
9.	Acetone	0.8783%	Not mention
10.	Methanol	2.4059%	Not mention
11.	Water soluble extractive	8.4672%	Not mention
12.	Total Alkaloids	0.286 %	Not less then 0.20%

Table 15 : TLC of Ashwagandha

S.No.	**Acetone Extract**		**Chloroform Extract**		**Methanol Extract**	
	Distance traveled by solute	Rf **Value**	**Distance traveled by solute**	Rf Value	**Distance traveled by solute**	Rf **Value**
1.	0.5	0.0769	0.6	0.15	1.5	0.214
2.	1.0	0.1538	1	0.25	2	0.285
3.	1.4	0.2153	2	0.5	2.8	0.4
4.	1.8	0.2769	2.4	0.6	3.2	0.457
5.	2.5	0.3846	3	0.75	3.6	0.514
6.	3.0	0.4615	3.4	0.85	4.5	0.642
7.	4.0	0.6153	3.9	0.975	5	0.714

8.	4.9	0.7538	-	-	5.8	0.828
9.	5.6	0.8615	-	-	-	-

CONCLUSION

The result drown were No foreign mater was determined. PH of churna was 5.80 in. The moisture content in drug power was 4.96 %. Total ash obtained was 7 %, acid insoluble ash was 0.15 %, water-soluble ash was 1.65 %, and water-soluble extractive was 16.48 %. Alcohol soluble extractive was 16.5 %, and Ether soluble extractive was 0.50 %.

Most of the analytical values drawn from the analytical study of various parameters of sam-ple drug are found according to the standards laid down in API.

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CORRESPONDING AUTHOR

Dr. Rajeev Kushwah

Bundelkhand Govt.Ayurvedic College & Hospital, Jhansi, Uttar Pradesh, India

Email: drajeev.kushwaha@gmail.com